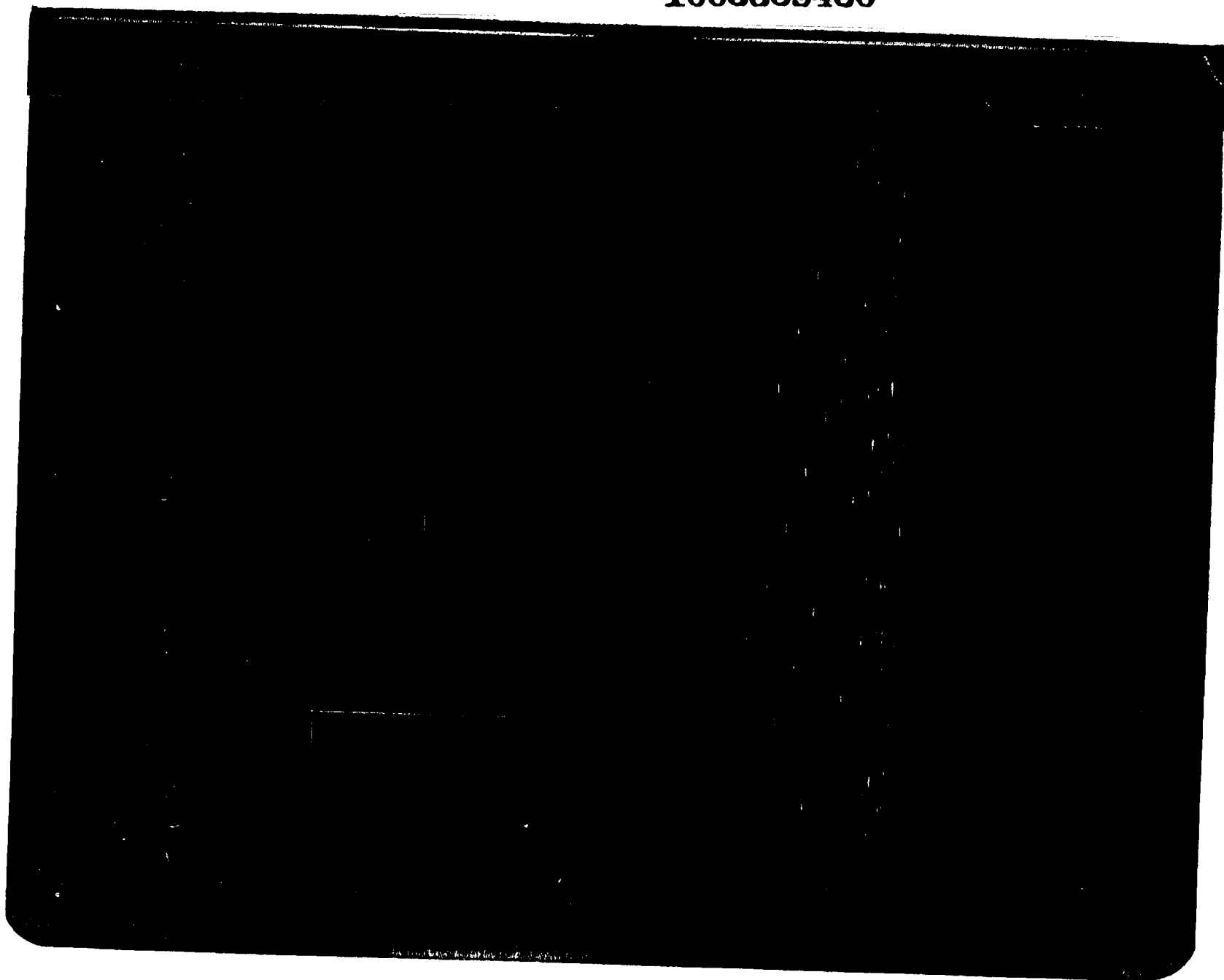


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PULMONARY

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#915-BRESNICK

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

June 22, 1973

Grant application No. 915

TO: The committee comprising Drs. Andervont, Gardner and Sommers

SUBJECT: Edward Bresnick, Medical College of Georgia, Augusta
New application No. 915
"Synthesis, Interaction with DNA, and Inactivation of the Epoxide
of 3MC in Lung"

History

This proposal originated as case #190 and application was encouraged (without commitment of course).

Application No. 915 requests \$40,650., plus two additional years.

Documents Submitted

Attached is application dated May 29, 1973. (13 pages)

Reprints of the six publications listed in the Bresnick vita (p. 9) were submitted, and will be forwarded to you on request.

Comment

An outside opinion seems unnecessary, but will be sought if you wish.

FWN:gh


F.W.N.

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Comm.

Dr. Andervont
Dr. Gardner
Dr. Sommers

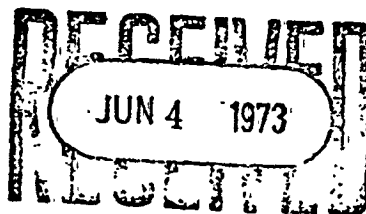
CARCINOGENESIS

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8985

Application for Research Grant.
(Use extra pages as needed)

Date: May 29, 1973



1. Principal Investigator (give title and degrees):
Edward Bresnick, Ph.D.
Professor and Chairman
2. Institution & address:
Medical College of Georgia
Gwinnett Street
Augusta, Georgia 30902
3. Department(s) where research will be done or collaboration provided:
Cell and Molecular Biology
4. Short title of study:
Genetic and Environmental Influences on the Synthesis, Interaction with DNA, and Inactivation of the Epoxide of 3MC in Lung
5. Proposed starting date: January 1, 1974
6. Estimated time to complete: 3 years
7. Brief description of specific research aims:
 - a. To ascertain the extent of formation of 3-methylcholanthrene (3MC)-11,12-oxide in lung tissue obtained from a series of mice which possess varying susceptibilities to polycyclic hydrocarbon-induced tumorigenesis
 - b. To determine the rate of detoxification of the epoxide of 3MC in lung tissue, i.e., the activity of epoxide hydrase, obtained from these mice.
 - c. To ascertain the extent of the repair process in lung tissue after incubation with polycyclic hydrocarbon and the effect of 3MC and its epoxide upon the radiation-induced repair mechanism in fibroblasts.
 - d. To ascertain the effects of prior exposure to 3MC upon the above parameters, i.e. what effect does 3MC administration have upon epoxide synthetase, hydrase, and the repair mechanism.
 - e. To attempt to correlate the above parameters with polycyclic hydrocarbon-induced lung tumorigenesis.

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8. Brief statement of working hypothesis:

2.

The remarkable spectrum of lung tumorigenesis among inbred strains of mice has suggested the involvement of genetics in susceptibility or resistance to this type of cancer. Furthermore, given a genetic susceptibility, environmental chemicals, i.e., polycyclic hydrocarbons, increase the numbers of tumors and decrease the latency time. It is the hypothesis of the present study that susceptibility may be related to a) the genetically-determined basal levels of epoxide synthetase, epoxide hydrase and the extent of the repair mechanism and b) the magnitude of the response to the prior administration of 'inducers', such as 3-MC. Those individuals who have low levels of the detoxifying enzyme, i.e., hydase, high levels of the synthetase and a reduced repair capability would be susceptible to lung tumorigenesis. Furthermore, the response to 'inducers' should involve increased synthetase activity relative to hydase and decreased relative repair capability. This hypothesis will be tested in mice that exhibit varying degrees of susceptibility to 3MC-induced pulmonary tumorigenesis.

9. Details of experimental design and procedures (append extra pages as necessary)

Although millions of humans are constantly exposed to an environment which is rich in polycyclic hydrocarbons through inhalation of cigarette smoke and exhaust products, only a small percentage will ultimately develop lung cancer. This observation implies the existence of populations of resistant and susceptible individuals. In the mouse, a similarly-remarkable spectrum in susceptibility to either spontaneous or polycyclic hydrocarbon-induced pulmonary tumorigenesis has been observed ranging from 100% incidence in some inbred strains to almost complete resistance.

In this regard, Andervont (1) and Shimkin (2) have reported on the comparative susceptibilities of a number of strains of mice to the induction of pulmonary tumors after administration of 1,2,5,6 dibenzanthracene and 3-methylcholanthrene (3MC), respectively. The results of these and of other studies have strongly implicated genetics as an important determinant in lung tumor development. In the studies cited above (1,2), it was also concluded that given a genetic capability, environmental factors would then play a prime role in the development of neoplastic disease. Thus, for example, the administration of 3MC to genetically-susceptible mice resulted in the appearance of more tumors and at an earlier time, i.e., shorter latent period.

It is the primary goal of this research proposal to understand the nature of the 'genetic susceptibility' and the manner in which 3MC can exert its effect. As a corollary, the mechanism of resistance of certain inbred strains of mice to polycyclic hydrocarbon-induced pulmonary tumorigenesis will be under study.

Kirschbaum Memorial Mouse Laboratory: One of the unique aspects of this proposal is the ready availability of numerous mouse model systems that have been developed over the past 30 years by Dr. A. G. Liebelt and that can now serve as the source material for the objectives set forth in this proposal.

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These mouse model systems were developed in the Kirschbaum Memorial Laboratory, a facility which was established at Baylor College of Medicine in 1958 following the death of the late Dr. Arthur Kirschbaum and which has been recently moved (in 1971) to the Medical College of Georgia. The mouse colony is still under the direction of Dr. Annabel Liebelt and consists of 17 different inbred strains of mice that have been maintained by brother-sister matings since their introduction into the colony, in some cases over 40 years ago. All strains are tested periodically for genetic homogeneity by reciprocal skin grafting techniques. In addition, all autopsies of all experimental and colony mice have been carried out either personally or under the direct supervision of either Dr. A. G. Liebelt or Dr. R. A. Liebelt for the past 15 years thereby assuring a uniform pattern of data collection. A computer program written in PLI language was initially developed at Baylor College of Medicine and is being continued here at the Medical College of Georgia to facilitate retrieval and analyses of available data now kept in record books.

The available mouse strains include:

Strain: A/Ki
Origin: Strong to Kirschbaum in 1944
Charac: mammary cancer incidence 80% in breeding ♀♀, 30% in virgins - enhancement after methylcholanthrene. Reticular tissue neoplasms, primarily histiocytomas, 4% in breeding ♀♀, 12% in ♂♂. Reciprocal skin grafts with Af/BiKi not accepted.
Inbreeding: F? + 66 (66 in this laboratory)

Strain: Af/BiKi
Origin: Bittner (who called it Ax) to Liebelt in 1961
Charac: mammary cancer less than 1% in breeding ♀♀ and leukemia less than 1%. Reciprocal skin grafts with A/Ki not accepted
Inbreeding: F61 by Bi after fostering and F24 in this laboratory

Strain: AKR/Ki
Origin: NIH to Ki in 1956
Charac: leukemia primarily lymphatic and involving thymus - 83% in ♀♀ at av. age 9 1/2 mo., 68% in ♂♂ at av. age 10 1/2 mo.; ♂♂ at av. age 10 1/2 mo., ♂♂ fight.
Inbreeding: F46 in this laboratory

Strain: BALB/cKi
Origin: Bittner to Kirschbaum 1952
Charac: mammary cancer less than 8% in breeding ♀♀ (primarily in one subline) - enhanced after methylcholanthrene - enhanced after MTA introduced. Leukemia less than 12% in ♀♀ (primarily in one subline) - enhanced after methylcholanthrene - enhanced after MTA introduced. Leukemia less than 12% in ♀♀ and less than 10% in ♂♂.
Reciprocal skin grafts with BALB/cKi accepted.
Inbreeding: F65 by Bittner and F31 in this laboratory.

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Strain: CBA/Ki
Origin: Strong (probably) - Kirschbaum 1941
Charac: mammary cancer less than 2% in breeding ♀♀. Leukemia less than 6% in ++, less than 3% in ♂♂. Hepatomas less than 2% ++, 9% ♂♂ at approx 1 1/2 years. Uniformly susceptible to 0.35 mg/gm BW goldthioglucose - 100% survival and obesity. Reciprocal skin grafts with CBA/StKi not accepted. Has retinal degeneration
Inbreeding: F? + 61

Strain: CBA/StKi
Origin: Strong to Liebelt 1963
Charac: mammary cancer 40% in breeding ♀♀. Reciprocal skin grafts with CBA/Ki not accepted. Does not have retinal degeneration
Inbreeding: F? + 37

Strain: CE/Ki
Origin: (Wooley?) to Kirschbaum 1952
Charac: mammary cancer 3% in breeding ♀♀, ovarian tumors less than 10%, reticular tissue neoplasms less than 3%
Inbreeding: F? + 37

Strain: C3H/BiKi
Origin: Bittner (Z) to Kirschbaum 1972
Charac: mammary cancer 83% in breeders, variable in virgins. Leukemia less than 0.5% in breeding ++, 14% in ♂♂. Hepatomas 0% in ++, 10% in ♂♂. Reciprocal skin grafts with C3H/BiB/Ki accepted
Inbreeding: F80 by Bittner and 41 in this laboratory

Strain: C3H/BiB/Ki
Origin: Bittner's Zb - C3H fostered on C57Bl. Bi to Kirschbaum 1956
Charac: mammary cancer 2% in breeding ++. Leukemia 9% in ++, 3% ♂♂. Hepatomas 2.5% ++, 12% ♂♂. Reciprocal skin grafts with C3H/BiKi accepted. Grafts with C3H/Crg1 not accepted
Inbreeding: F117 (83 by Bi, 34 by Ki)

Strain: C57BL/Ki
Origin: Bittner to Kirschbaum 1951
Charac: mammary cancer 0% in breeding ++. Reticular tissue neoplasms less than 10% in ++, less than 4% in ♂♂. Some hydrocephalus
Inbreeding: F? + 45

Strain: DBA/2Ki
Origin: Bittner to Kirschbaum 1944
Charac: mammary cancer 66% in breeding females, 30% virgins. Leukemia 6% ++, 8% ♂♂. Both types of cancer enhanced with methylcholanthrene.
Inbreeding: F? + 56

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Strain: DBA/2_fKi
Origin: Bittner fostered DBA/2 on Zb in 1945 - to Kirschbaum
Charac: mammary cancer less than 3% in breeders; leukemia 10% ♀♀, 2% ♂♂
Inbreeding: F? + 49

Strain: DBA/2_{ff}Ki
Origin: Kirschbaum fostered DBA/2f (low cancer) on C3H (+) in 1945
Charac: mammary cancer 76% in breeding females, 43% in virgins.
Leukemia 5% females, 4% males
Inbreeding: F? + 51

Strain: FB/Ki
Origin: Kirschbaum 1942 - [(A x F) X F] x [(A x F) X F] - FA
1952 - [(NH x FA) x FA] x FA = FB
Charac: reticular tissue neoplasms approx 50%; some myeloid leukemia
Inbreeding: F36

Strain: IS/BiKi
Genet.: no chinchilla as I^b and I
Origin: Strong 1950 to Bittner - to Liebelt 1961
Charac: resistant to mammary cancer - do not propagate MTA. Trans-
mit inducing hormonal pattern for mammary cancer
Inbreeding: F74 by Bittner and F19 in this laboratory

Strain: NH/Ki
Origin: Kirschbaum 1942
Charac: Low tumor incidence; nodular hyperplasia and adrenal adenomas
in at least 10% of old mice--gonadectomy at 6 weeks enhances
adenomas. Breeding ceases at approx. 9 months. Some obesity.
One subline losing piebald and becoming albino
Inbreeding: F70

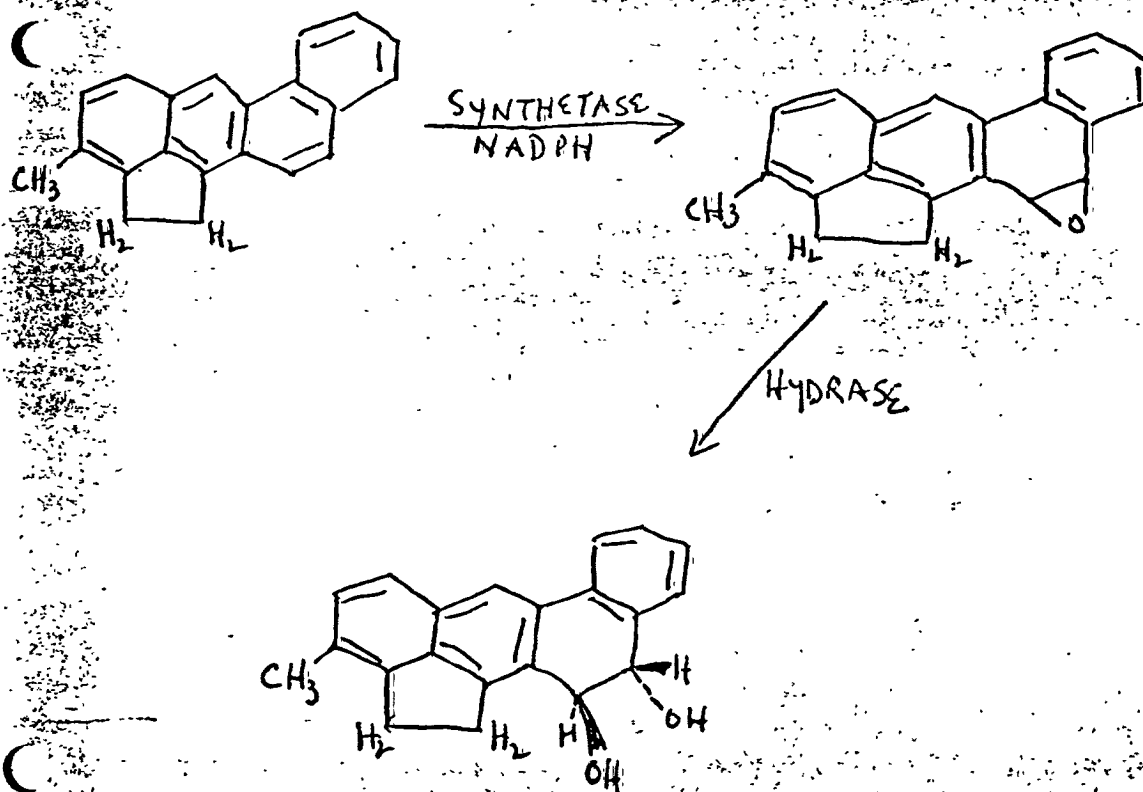
Strain: YBR/Ki
Genet: A^a a
Origin: Heston to Liebelt 1958
Charac: amyloid at least 50% in males and females with A^a or aa.
Obesity in F1 hybrids between A^a and other strains. Low tumor.
Inbreeding: F65 (He) + 29 (Ki)

These strains include those which are highly susceptible to pulmonary
tumors, Balb/c, NH, A, CBA; moderate to low incidence, DBA, C3H, FB,
IS, YBR; and low to no incidence, C57Bl, AKR.

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Aryl Hydrocarbon Hydroxylase and 3MC Metabolism: The administration of 3MC to rodents or its addition to *in vitro* systems is accompanied by the appearance of a number of metabolites including the 1 (or 2) hydroxy, 1 (or 2) keto, cis and trans 1,2 dihydroxy, cis and trans 11,12 dihydro 11,12 dihydroxy, and 11,12 epoxy derivatives (3-6). The biotransformation of 3MC to the metabolites is catalyzed by the microsomal NADPH-dependent enzyme, aryl hydrocarbon hydroxylase. The latter enzyme system is present and "inducible" *in vivo* by polycyclic hydrocarbons and other substances in a number of tissues, including lung. However, the basal and "inducible" levels of aryl hydrocarbon hydroxylase vary greatly among the various tissues, different species and various strains of a mammalian species; genetic factors apparently influence the level of control enzyme as well as the extent of the response (induction) to environmental influences, i.e., polycyclic hydrocarbons (4,7).

Aryl hydrocarbon hydroxylase is a complex system which catalyzes diol formation presumably through a reactive intermediate, an epoxide. The sequence of reactions leading to the formation of the trans 11,12 dihydro-11,12-dihydroxy 3MC from 3MC is indicated below. The product of the 1st reaction is the 11,12-oxide catalyzed by epoxide synthetase; diol formation proceeds through the catalysis of epoxide hydrazase.



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It was Boyland (8) who initially proposed in 1950 that epoxides may be formed from aromatic ring structures as intermediates in the transition to diols and phenols. Since that time, epoxides have been reported as intermediates in the metabolism of polycyclic hydrocarbons (9-11). The formation of epoxides (particularly at the 11,12 double bond or K-region) has great import in view of the production of mutational changes i.e., "malignant" transformation, upon addition to cultured mammalian cells (12). The trans diol, on the other hand, would represent a less carcinogenic substance. Consequently, not only is the rate of synthesis of the K-region epoxide of 3MC important, but the subsequent rate of hydration must be given prime consideration. The intracellular quantity of the reactive epoxide will therefore be a function of the levels of epoxide synthetase and hydrase enzymes.

As noted previously, aryl hydrocarbon hydroxylase activity is markedly induced in tissues by prior administration of 3MC. It is not clear, however, whether this induction is the result of increased activity of the epoxide synthetase, hydrase, or both these components.

Assay for 3MC Epoxide Hydrase Activity in Lung: Because of the importance of this enzyme in the inactivation of 3MC, we propose to assay 3MC epoxide hydrase activity in microsomal preparations of lungs obtained from mouse strains which are susceptible to 3MC-induced pulmonary neoplasms, i.e., Balb/c, NH, A, CBA; from mouse strains which are only moderately susceptible, i.e., DBA, C3H, FB, I^S, YER; and from those which are resistant to carcinogenesis, i.e., C57Bl, AKR.

The basic assay for epoxide hydrase depends upon separation of the trimethylsilyl (TMS) derivative of the trans diol from that of the 11,12 oxide (the latter is decomposed to the 11 or 12 TMS derivative). Since the method is in press (13,14) and has not appeared yet, it will be described in some detail. Furthermore, preprints are included in the Appendix.

The 11,12 oxide as well as the cis and trans diol derivatives of 3MC were prepared by the method of Sims (5). The trimethylsilyl derivatives were formed according to the following procedure: To 1 mg of compound was added 0.4 ml of Tri-SylR (Pierce Chemical Company) and after the resultant mixture was heated at 60° for 30 min, 0.2 ml of N,O-bis-TMS-trifluoroacetamide (Pierce Chemical Company) was introduced. The solution was brought to a final concentration of 1 mg polycyclic hydrocarbon per ml by the addition of acetonitrile and stored overnight at 5°. The derivatives were stable under these conditions for 2 days. All steps were conducted under conditions of reduced light.

The gas chromatography was conducted in a Nuclear Chicago Model 5000 instrument containing a U-tube glass column, 4 mm x 6 ft, in which was packed 100-120 mesh gas chrom Q coated with 3% OV-17 (Applied Science

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Laboratories). The temperature was programmed from 220°-320° at 30/min. The mobilities of the substances were expressed as methylene units in comparison to the retention of known hydrocarbon standards (Table I). The identities of the TMS derivatives were established by mass spectrometry.

TABLE I
Retention of 3MC Derivatives on OV-17

<u>TMS Derivative of</u>	<u>Methylene Units</u>
cis 11,12 dihydro 11,12 dihydroxy 3MC	32.54
trans 11,12 dihydro 11,12 dihydroxy 3MC	29.63
11,12 oxide	36.00

Although the hydration of styrene oxide has been employed as the basis for the hydrazine assay in previous work (15), this reaction might not reflect an accurate assay of the formation of trans dihydrodiol derivatives of polycyclic hydrocarbons. The assay just described is specific for 3MC. The gas chromatographic assay also has the following advantages: 1) it employs relatively simple substrates and can be employed for all aromatic epoxides. 2) difficulties associated with radioactive contamination, tritium exchange reactions, and differential isotope effect are avoided. 3) the assay is extremely sensitive and can detect less than 0.2 nmoles of diol.

Following incubation of 3MC-11,12 oxide with the tissue preparation, the products are extracted into ethyl acetate. The latter was evaporated and the TMS derivatives were prepared.

The major enzyme activity in liver is associated with the microsomal fraction, although the 600-20,000 x g pellet fraction possessed a specific activity which was approximately 30% of that seen with the 100,000 x g pellet (4.0 vs 11-12 nmoles diol formed per mg protein per 10 min.). A good deal of the former activity may be associated with microsomal contamination. No activity could be found in the supernatant fraction.

The reaction with liver appeared linear for up to 10 min and consequently, the latter time was chosen as a part of the assay conditions. A study of velocity vs substrate concentration of the reaction indicated that 0.44 mM epoxide (50 µg) was sufficient to saturate the system; a calculated K_m for this substrate was 2.8×10^{-4} M. Enzyme activity was also directly proportional to the protein concentration of liver microsomes, i.e., to 10 mg protein.

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Liver microsomes possessed the greatest amount of activity whereas microsomes obtained from kidney and lung appeared to be 25% and 5% as active, respectively. No demonstrable enzyme activity could be detected in microsomes obtained from either spleen or small intestine. Finally, as reported by Oesch and colleagues (16) for the hydration of styrene oxide, equimolar quantities of 1,1,1-trichloropropene oxide (TCPO) completely inhibited the formation of the diol from 3MC-epoxide.

This series of experiments will allow us to examine the basal levels of 3MC epoxide hydase in lung microsomes from the various mouse strains.

3MC Epoxide Synthetase Activity: This enzyme is a key in the activation of 3MC to the 11,12 oxide. Although in previous studies, its activity was semi-quantitatively measured by employing labeled 3MC in the presence of a bank of epoxide, we propose to modify this assay as follows:

Epoxide synthetase can be measured by employing the same assay conditions as described for the hydase but with 3MC as substrate and with the inclusion of NADPH-generating system. In addition, an inhibitor of hydase, TCPO, will be added to block the subsequent hydration of the epoxide. Under the conditions of the assay, 3MC is not derivatized to any TMS compound and the 11,12 oxide is converted to the 11 or 12 TMS derivative.

In addition, we intend to modify the assay conditions for synthetase activity so as to achieve greater sensitivity by employing the electron capture detector. Toward this end, 3MC will be incubated with lung microsomes, TCPO and a NADPH-generating system. Appropriate controls, i.e., heated or no microsomes, as well as no NADPH, will run simultaneously. The metabolic products will be extracted as described in the hydase section and derivatized with perfluorobenzoylchloride. Under these conditions the 11,12 oxide is converted to the 11 or 12 phenol which is coupled to form the perfluorobenzoate ester. The latter can be determined with great sensitivity by gas chromatographic techniques with the electron capture detector. It may be necessary to 'clean' up the derivative first by thin layer chromatography on silica gel with benzene as the developing solvent. These studies are presently underway.

After establishing the validity of this synthetase assay, we will then be able to catalog the 3MC epoxide synthetase activity in lung microsomes obtained from the various mouse strains.

Induction of microsomal enzyme activity: Having established the basal levels of the synthetase and hydase in lung tissue obtained from susceptible, moderately-susceptible and resistant mice (to polycyclic hydrocarbon carcinogenesis), we will then examine the response

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of these enzymes to 3MC administration. The latter will be injected in corn oil i.p., into male mice, 6-8 weeks of age, at a level of 20-100 mg/kg body wt and the synthetase and hydase activities will be ascertained in lung microsomes as described above, at various times after administration of the polycyclic hydrocarbon. Suitable control mice of the same age and sex will be injected with the vehicle alone and enzyme activities determined. In this manner, we will be able to determine if prior exposure to 3MC can influence the levels of either the synthetase, hydase or both in lung tissue obtained from the various mouse strains.

In addition to 3MC, we propose also to examine the effects of other 'inducers', e.g., β -naphthoflavone (370 μ moles/kg i.p. in corn oil), phenobarbital (75 mg/kg, twice daily for 3 days, i.p.), and pregnenolone 16 α carbonitrile (10 mg/kg, 5 times at 12 hr intervals, i.p.) upon synthetase and hydase activities in lung tissue at appropriate intervals after drug treatment.

Extent of the Repair Mechanism in Lung: Mammalian cells have a remarkable capacity to repair by "unscheduled DNA synthesis" its genetic material which might have been damaged by a variety of agents including UV irradiation, carcinogenic and non-carcinogenic alkylating agents. Consequently, this repair mechanism represents a fundamental life process which may have important implications in chemical carcinogenesis. In fact, a reduced level of DNA repair synthesis has been noted in cells from patients with Xeroderma pigmentosum, a condition manifested by increased sensitivity to UV irradiation and increased skin cancer (17). Since it has been postulated that all carcinogens interact covalently with macromolecules, including DNA (18), the extent to which a tissue can repair this 'hit' may in part determine its susceptibility to chemically-induced tumorigenesis. We propose to study the repair process initially in fetal lung explants which have been subjected to 3MC or its 11,12 oxide under culture conditions. The latter will be similar to those employed with fetal liver as reported previously (3,4); we have shown that lung can be used under these conditions. The explants will be preincubated with 3MC or the 11,12 oxide for varying periods of time and then incubated with ^3H -thymidine in the presence of hydroxyurea (10^{-2} M). Under these conditions, S-phase DNA synthesis is completely abolished and only unscheduled repair continues. The relative rates of ^3H -thymidine incorporation into DNA will be determined by standard methods, as have been used in this laboratory, in the presence and absence of hydroxyurea after preincubation with or without 3MC or its oxide. Additional purification of the isolated DNA may be required by isopycnic centrifugation in CsCl as outlined by Flamm (19). From these results, we should be able to obtain a first approximation as to the extent of the repair mechanism in lung tissue from various mouse strains.

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The objective of these studies is to determine the extent of the repair system after addition of 3MC and its 11,12 oxide to fetal lung preparations obtained from various mouse strains.

The extent of binding to lung DNA of ^3H -labeled 3MC, which had been injected subcutaneously into male mice of different strains, will also be ascertained at various times after administration of the polycyclic hydrocarbons. We should be able to obtain some information about the rate of binding under these *in vivo* conditions as well as the rate of disappearance of the label from the lung DNA over the course of time.

It will be of importance to determine a) the effects of prior administration of 'inducers' e.g., β -naphthoflavone, upon the *in vivo* binding of labeled 3MC to lung DNA and the disappearance of label from the latter b) the effects of addition of inducers upon the level of "unscheduled DNA synthesis" in the fetal lung explant system.

Effect of 3MC and the 11,12 Oxide on the Repair Mechanism: It is possible that some of the action of 3MC (or its metabolites) may be due to interference with the repair mechanism. Accordingly, we propose to use the fibroblast system for this phase of the investigation. Human fibroblasts (originally obtained from a prepucce) are routinely grown in culture in this Department. We propose to investigate the "unscheduled synthesis of DNA" after UV irradiation of these cells (10 erg/min²/sec by 15 watt germicidal lamp) followed by their incubation with ^3H -thymidine in the presence of 10 mM hydroxyurea, largely as outlined by Brandt et al (20). After establishing the baseline levels for repair with these cells 3MC or its 11,12 oxide will be added during the repair phase and the extent of incorporation will be determined.

The research outlined in this proposal represents approximately 3 years effort. The first priority will be given to establishing the base line levels of synthetase and hydase in lung microsomes obtained from various mouse strains. These studies will be followed by the induction experiments, i.e., after administration of 3MC or β -naphthoflavone to the mice, and finally, the series on repair will be conducted later.

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References

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The principal investigator has approximately 1500 sq ft of laboratory space in the Sanders Research and Education Building in addition to access to cold rooms, culture rooms (with lamilar flow hoods) and the mouse colony (Kirschbaum Memorial Mouse Laboratory). The major items of equipment presently in the laboratory include: liquid scintillation spectrometers; microscopes; water-jacketed CO₂ incubators; all types of centrifuges; electrophoretic and chromatographic equipment; spectrophotofluorometers and fluorimeters; spectrophotometers; Nuclear gas chromatograph with FID and Beckman GC-65 with electron capture. In addition, we have access to a Zeiss electron microscope. In short, we have all the necessary equipment and space to accomplish the aims of this research proposal.

11. Additional facilities required:

No additional facilities required.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

See Enclosure

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

4.

14. First year budget:

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s)
even if no salary requested)Edward Bresnick, Prin. Invest.
Terry A. Stoming, Ph.D.
Annabel G. Liebelt, Ph.D.

% time Amount

25 REDACTED
100
10Technical
Research Assistant II

100 REDACTED

REDACTED

Fringe Benefits

Sub-Total for A REDACTED

B. Consumable supplies (by major categories)

Chemicals
Radioisotopes
Laboratory glassware2,000
2,000
2,000

Sub-Total for B 6,000

C. Other expenses (itemize)

Boarding charges for mice
Travel for 2 to 1 national meeting
Maintenance contracts (for centrifuges, etc.)3,000
800
500

Sub-Total for C 4,300

Running Total of A + B + C 35,348

D. Permanent equipment (itemize)
None

Sub Total for D

E 5,302

Total request 40,650

E. Indirect costs (15% of A+B+C)

3. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2	REDACTED	6,000	4,300	-	5,478	41,997
Year 3		6,000	4,300	-	5,714	43,806

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16. Other sources of financial support

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None at this time			

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Not at this time			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

James A. Blissit, Vice President/Treasurer

Mailing address for checks

Medical College of Georgia

Augusta, Ga. 30902

Principal investigator

Typed Name Edward Bresnick, Ph.D.

Signature *Edward Bresnick* Date 5/30/73

Telephone 404-724-7111 Ext. 8721

Area Code Number Extension

Responsible officer of institution

Typed Name Robert A. Liebelt, M.D., Ph.D.

Title Provost
Signature *Robert A. Liebelt* Date 6/1/73

Telephone 404-724-7111 Ext. 8863

Area Code Number Extension

1003539448

6

CURRICULUM VITAE

EDWARD BRESNICK

REDACTED

Social Security Number:Date and Place of BirthSex: MaleMarital Status:REDACTED
REDACTED

Education: St. Peter's College, B.S., magna cum laude,
Fordham University, M.S., REDACTED
Fordham University, Ph.D.,

Positions: Research Associate, University of Texas Medical Branch, 1957-1958
Research Biochemist, Wellcome Research Labs., 1958-1961
Senior Research Biochemist, above, 1959-1961
Assistant Professor of Biochemistry, Baylor University College of
Medicine, 1961-1963
Assistant Professor of Pharmacology, Baylor University College of
Medicine, 1963-1965
Associate Professor of Pharmacology, Baylor University College of
Medicine, 1965-1968
Professor of Pharmacology, Baylor University College of Medicine,
1968-1971
Professor and Chairman, Department of Cell and Molecular Biology,
Medical College of Georgia, 1971-present
Acting Dean, School of Graduate Studies, Medical College of
Georgia, 1972-present

Scientific Societies:

REDACTED

Honorary Societies:

Phi Lambda Upsilon
Sigma Xi

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7
Awards: Lederle Medical Faculty Award, 1966-1969

National Committees:

Member of the Pharmacology-Toxicology Review Committee for the National
Institute of General Medical Science,
1968-1972.

Other Honors:

Award for "Outstanding Basic Science Faculty Member," 1966.
Award for Excellence in Teaching, by Phi Chi Medical Fraternity, 1968
President, Texas Medical Center Research Society, 1968
Elected to Represent Basic Sciences on the Executive Faculty, 1969

Functions in the University:

Medical School (Baylor):

Elected Representative on the Executive Faculty, 1969
Member, Medical Admissions Committee
Chairman, Subcommittee for Minority Admissions
Member, Radioisotope Committee
Member, Faculty Research and Fellowships Support Committee
Member, Curriculum Committee
Vice-chairman, Student Affairs Committee
Member, Subcommittee of Faculty Advisors

Graduate School (Baylor):

Member, Graduate Executive Committee
Chairman, Cell Biology Committee
Member, Graduate Admissions Committee
Member, Course and Curriculum Committee
Member, Scholarships Committee
Director of Graduate Studies, Department of Pharmacology, 1966-1968

Medical School (Medical College of Georgia):

Member, Senior Administrative Group
Member, Executive Committee
Member, Phase I-II Curriculum Committee
Member, Long Range Planning Committee
Member, Physiology Search Committee
Member, Pathology Search Committee
Member, Grants and Contracts Advisory Committee
Member, Board of Directors of the Augusta Radiation Therapy Center,
Augusta, Georgia
Member, Professional Services Committee of the Augusta Radiation Center
and Tumor Institute, Inc.
Member, Administrative Council
Member, Health Communications Advisory Committee
Member, Academic Council of the School of Nursing
Elected Faculty Representative to the Student Council (1973) of the
Medical College of Georgia.

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8

Graduate School (Medical College of Georgia:

Member, Graduate Council Committee

Editorial Work: Journal of Pharmacology and Experimental Therapeutics,
Field Editor.

Biochemical Pharmacology, Editor.

Member, Board of Honorary Editorial Advisers of
Biochemical Pharmacology

Mentioned in:

American Men of Science
World Who's Who in Science
Who's Who in America
Dictionary of International Biography
Community Leaders of America

1003539451

9

Publications:

1. Yee, M. and Bresnick, E.: Effects of administration of 3-methylcholanthrene upon the 2M NaCl-extractable chromatin proteins in rat liver. *Mol. Pharm.*, 7:191-198, 1971.
2. Bürki, K., Seibert, R. A., and Bresnick, E.: Induction of Benzpyrene Hydroxylase Activity in Fetal Rat Liver Explants: Metabolism of 3-Methylcholanthrene and Differential Effects of Its Derivatives of Benzpyrene Hydroxylase Activity *Biochimica et Biophysica Acta*, Vol. 260, pp. 98-109 (1972).
3. Bürki, K. and Bresnick, E. : Cellular Responses to Methylcholanthrene: The Role of Benzpyrene Hydroxylase in the Binding of Polycyclic Hydrocarbon to Cytosol Macromolecules in Fetal Rat Liver Explants. *Arch. Biochem. & Biophys.*, Vol. 152, pp. 574-589, 1972.
4. Bürki, K., Liebelt, A. G. and Bresnick, E.: Induction of Aryl Hydrocarbon Hydroxylase in Mouse Tissues from a High and Low Cancer Strain and their F₁ Hybrids. *J. Nat'l Cancer Inst.* Vol. 50, pp. 369-380, 1973.
5. Stoming, T. A, Knapp, D. R., and Bresnick, E.: Gas Chromatographic Separation of the K-Region Epoxide and of the Cis and Trans 11,12 Diol Derivatives of 3-Methylcholanthrene. *Life Sci.* Vol. 12, Part II, pp.425-429, 1973.
6. Bresnick, Edward, Stoming, T. A., A Gas Chromatographic Assay for Epoxide Hydrase Activity with 3-Methylcholanthrene-11,12-Oxide Science, accepted.

1003539452

Terrance A. Stoming
Department of Cell and Molecular Biology
Medical College of Georgia
Augusta, Ga. 30902

Telephone: 724-7111 (Ext. 8744)

Personal:

REDACTED

Professional Objective: Teaching and conducting research in the area of medicinal and biochemical organic chemistry at the university level.

Education: REDACTED, Purdue University

Major: Pharmacal Sciences

Grade index: 5.10/6.00

REDACTED

Purdue University

Major: Synthetic Medicinal Chemistry

Ph.D., January R Purdue University

Major: Synthetic Medicinal Chemistry

Grade index: 5.43/6.00

Experience

November, 1971 - present: Medical College of Georgia
Department of Cell and Molecular Biology
Post-doctoral research
Augusta, Georgia

1969 to November, 1971: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Graduate Research. The design and synthesis of potential histamine agonists.

1966 to 1969: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Graduate Research. The Attempted Synthesis of a Possible Cyclopentanoterpenoid Precursor.

1965 to 1966: Purdue University
School of Pharmacy and Pharmacal Sciences
Department of Medicinal Chemistry
Lafayette, Indiana 47907
Undergraduate research. The Synthesis of Ring Substituted α -isopropylaminophenones.

Fellowships
1970 to 1971

American Foundation for Pharmaceutical Education,
E. Mead Johnson Memorial Fellow. Teaching
Assistant, Biochemistry.

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Page 2 continued

Fellowships
1969 to 1970:

American Foundation for Pharmaceuti-
cal Education
Teaching Assistant, Medicinal
Chemistry

1966 to 1969:

N.D.E.A. Title IV Research Fellow

1965 to 1966:

N.S.F. Undergraduate Research Fellow

Military Service:

None

Present Draft Status: 3-A

Professional and Honor Societies:

REDACTED

Publications:

The Design and Synthesis of Potential Histamine
Agonists (Awaiting Pharmacological Evaluation)
will consist of two (2) publications.

Gas Chromatographic Separation of the K-region
epoxide and of the Cis- and Trans-11,12-Diol
Derivatives of 3-Methylcholanthrene. Life
Sciences II 12, 425 (1973).

A Gas Chromatographic Assay for Epoxide Hydrase
Activity with 3-Methylcholanthrene-11,12-Oxide
Science, in press.

1003539454

CURRICULUM VITAE

ANNABEL GLOCKLER LIEBELT, B.A., M.S., Ph. D.Date and place of birth:

REDACTED

Children:Social Security Number:

REDACTED

REDACTED

Education:Public schools in Washington, D. C. - Calvin Coolidge High.
Western Maryland College, Westminster, Maryland, RMajor: Biology. Minors: English, Philosophy
University of Illinois College of Medicine, Chicago, IllinoisR Major: Anatomy, Advisor: Dr. Arthur Kirschbaum
Thesis: Homoplastic Skin Grafting in Inbred MiceBaylor University College of Medicine, Houston, Texas, R
Ph. D. Major: Anatomy, Minor: Microbiology. Advisors:
Dr. Arthur Kirschbaum (deceased May 28, 1959) and
Dr. John J. Trentin. Thesis: Transplantation of Normal
and Neoplastic Tissues in Inbred Mice Differing at "Weak"
Histocompatibility Loci.Positions:Biologist - National Cancer Institute, Department of Pathology,
Bethesda, Maryland, October 1949 to October 1952.
Dr. Harold Stewart.Teaching and Research Assistant, Department of Anatomy,
University of Illinois College of Medicine, Chicago, Illinois.
Also supervisor of mouse colony and experimental projects,
November 1952-1954, Dr. Arthur Kirschbaum.Teaching and Research Assistant, Department of Anatomy,
Baylor University College of Medicine, Houston, Texas.
Also supervisor of mouse colony and research projects,
August 1954 to 1958.Instructor in Anatomy, Baylor University College of Medicine, 1958-1962
Assistant Professor, Baylor University College of Medicine, 1962-1967.
Associate Professor, Baylor University College of Medicine, 1967-1971.
Associate Professor, Department of Cell and Molecular Biology, Medical
College of Georgia, Augusta, Georgia, 1971 - present.

1003539455

Director of Kirschbaum Memorial Laboratory (Mouse Colony
with maintenance of 17 inbred strains of mice used for research
and teaching).

Societies:

REDACTED

Honors and Awards:

Invited Speaker - Carcinogenesis, A Broad Critique, March 1966.
Twentieth Annual Symposium in Fundamental Cancer Research,
M.D. Anderson Hospital and Tumor Institute

Research Interests:

1. Factors involved in transplantation of normal and neoplastic tissues - genetic, hormonal, immunologic.
2. Host-tumor interrelationships in mice with spontaneous, induced, and transplanted tumors - hormones, plasma proteins and other blood parameters, extramedullary hematopoiesis, viruses, development of other disease, and aging,
3. Genetic, viral and hormonal aspects involved in normal development, preneoplasia and neoplasia of the mouse mammary gland - induction and therapy of tumors.
4. Etiologic factors in spontaneous and induced leukemias in inbred mice and hybrids - genetic, hormonal, viral, chemical and physical.
5. Neuroendocrine mechanisms and neoplasia - cancer of endocrine organs and their target tissues - and relationship to obesity, hypothalamic lesions, ablation of organs, administration of exogenous hormones.
6. Biology and cytology (histochemistry and electron microscopy) of various mouse neoplasms, including mammary, ovarian, adrenal, pituitary, and liver tumors, melanomas, and neoplasms of the reticular tissue. Certain biochemical parameters and their correlation to morphological alterations.

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#906 - BROOKS

1003539457

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

May 23, 1973

Grant application No. 906

TO: The committee comprising Drs. Loosli, Sommers and Wyatt

SUBJECT: Robert E. Brooks, Ph.D., University of Oregon Medical School
New application No. 906
"Morphological Studies on Induced Pulmonary Emphysema in Rabbits"

History

In November, 1972 a preliminary inquiry was received from Brooks and Moore. It was staff judgment to invite an application without consulting the then Planning Committee.

Subsequently Brooks wrote for information about smoke exposure devices. Attached is a copy of Dr. Kreisher's reply dated November 30, 1972.

Application #906 requests \$14,658 plus two additional years.

Document Submitted (attached)

Application dated March 28, 1973.

Comment

In view of strong SAB competence in this area of investigation, no outside opinions have been sought.

FWN:gh

Enclosure


F.W.N.

1003539458

Dr. Loosli

Dr. Sommers THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

Dr. Wyatt

110 EAST 59TH STREET
NEW YORK, N. Y. 10022

Application For Research Grant

APR 10 1973

Date: March 28, 1973

1. Name of Investigator(s): (include Title and Degrees)

Robert E. Brooks, Ph.D., Associate Professor of Pathology

Richard D. Moore, M. D., Professor and Chairman, Department of Pathology

2. Institution & University of Oregon Medical School

Address: 3181 S. W. Sam Jackson Park Road
Portland, Oregon 97201

3. Short Title of Project:

Morphological Studies on Induced Pulmonary Emphysema in Rabbits

4. Proposed Starting Date: July 1, 1973

5. Anticipated Duration of this Specific Study: Three years

6. Brief Description of Objectives or Specific Aims:

Spontaneous emphysema in old rabbits has been reported by two groups of investigators. Emphysema also occurs in rabbits as a sequela produced by intravenous injection of Freund's adjuvant (Moore, R. D., unpublished observations).

The objectives of the proposed study are: 1) the careful, morphological documentation of changes that occur in rabbit lungs following intravenous injection of complete Freund's adjuvant; 2) morphological examination of lungs from rabbits which inhaled whole cigarette smoke and gas-vapor phase from cigarette smoke before, during, or after development of pulmonary emphysema due to intravenous injection of complete Freund's adjuvant; and 3) morphological comparison of lungs from the above two experimental groups with lungs of rabbits treated either with compounds that increase or decrease the lung macrophage response to circulating complete Freund's adjuvant or with compounds which produce primary injury to the alveolar capillary circulation.

On the basis of what is learned from these studies, we expect to be able to determine if cigarette smoke (whole or gas-vapor phase) affects the onset, development or course of adjuvant-induced emphysema in the rabbit. And, if it appears that cigarette smoke does affect this animal model, whether the effect is likely to be due to action on alveolar macrophages, action on the pulmonary microcirculation, or on both.

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7. Give a Brief Statement of your Working Hypothesis: We hypothesize that adjuvant-induced emphysema of the rabbit will provide a profitable animal model for the study of the relation of cigarette smoke inhalation to pulmonary emphysema.

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

Appended.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

Appended.

10. Additional Requirements:

A suitable method for providing cigarette smoke inhalation to rabbits will have to be determined. We will need to purchase, for the second year of this project, a Walton Horizontal Smoke Exposure Machine (or equivalent). We have been in correspondence with Dr. John H. Kreiser, of the Council, regarding the Walton smoke machine. During the first year of the project, we will need to obtain advice from various sources and possibly carry out pilot studies on rabbits in order to work out procedural problems.

11. Biographical sketches of all principal and professional personnel (append)

Appended.

12. List of publications: (Five most recent as pertinent) (append)

Appended.

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3.

13. Budget: (1st year)

A. Salaries (Personnel by names)

Professional

Robert E. Brooks, Ph.D.

% time

15%

Amount

Richard D. Moore, M. D.

10%

Technical

Research Assistant (to be hired)

100%

REDACTED

*@ \$600/mo + 13% payroll assessment
(\$7200 + \$936)

Sub-Total

REDACTED

B. Consumable Supplies (list by categories)

Laboratory chemicals: tissue fixatives, tissue
embedding materials, stains, alcohols

500

Microtomy supplies: glass for microtome knives, specimen grids

200

Photographic supplies: plates, developers, fixers, papers

700

Sub-Total

1,400

C. Other Expenses (itemize)

Rabbits, purchase, about 70 animals @ \$5.00/animal

350

Animal care for rabbits for entire first year

2,300

1 only diamond microtome knife, 3.00 mm size

560

Sub-Total

3,210 12746

D. Permanent Equipment (itemize)

None

E. Overhead (15% of A + B + C)

1,912 1267

Total

14,658

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	REDACTED	\$1,500	\$2,500	\$2,500	\$1,881	\$16,924
Year 3		\$1,500	\$2,500	---	\$1,943	\$14,893

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It is understood that the applicant and institutional officers
in applying for a grant have read and found acceptable
the Council's "Statement of Policy Containing Conditions
and Terms Under Which Project Grants Are Made."

Signature

Director of Project

Signature

Business Officer of the Institution

Telephone

Telephone

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Electron Microscopic Study of Lung Alveolar Surface	Medical Research Foundation of Oregon	\$2,000	3-1-72 through 5-31-73

Pending

Development of an Electron Microscopy Program to Aid in Cancer Diagnosis	Milheim Foundation for Cancer Research	\$9,669	7-1-73 through 6-30-74
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8. EXPERIMENTAL DESIGN AND PROCEDURES

The first year of the proposed research will be primarily concerned with establishing a baseline of development of adjuvant-induced emphysema in the rabbit.

Rabbits will be prepared by the injection of 1 ml of complete Freund's adjuvant (CFA) by way of the marginal ear vein. For this study 5- to 6-month-old rabbits of both sexes will be used. Animals will be killed, by Nembutal injection, at weekly intervals up to 12 weeks following injection of CFA. Three animals will be used for each time point. After 12 weeks, rabbits will be sacrificed at three week intervals, up to 24 weeks. Untreated rabbits will be sacrificed at 6, 12, 18 and 24 week intervals. Six additional experimental and four additional control animals will be carried along to replace any rabbits that die during the experimental period of 24 weeks. Therefore, a total of 70 animals will be used during the first year of the project.

When the chest of each animal is opened at time of sacrifice, the great vessels of the heart will be clamped, the trachea exposed and cut, and the lungs allowed to collapse. A glutaraldehyde fixative solution will be introduced into the lungs through the trachea and the lungs allowed to expand with fixative until they fill the chest cavity. The trachea is tied off and the lungs removed from the chest and immersed in a container containing the same fixative. Two to 4 hours later, small pieces of peripheral lung are removed and postfixed with osmium tetroxide solution for electron microscopy. The following day, slices of remaining lung are cut about 2 mm thick with a special double-bladed knife or by meat slicer and the resultant slices examined on a lighted box by dissecting microscope for "subgross" morphology. Some of these slices and other thicker pieces are also prepared in the usual way for subsequent light microscopic examination.

Lung tissue from each rabbit will be studied at the gross, subgross, light and electron microscopic levels. Photographic and descriptive records will be made and analyzed in terms of tissue reactions.

Earlier studies have shown that lung tissue reaction to CFA consists mainly of exudation of macrophages into the air spaces and granuloma formation within the tissue of the lungs. Subsequent resolution of the exudate and granulomas leads to alveolar wall destruction. The ultra-structural events related to this alveolar wall destruction have not been followed closely. One of the main purposes of this proposed project is the detailed description of fine structural changes occurring in the lungs of the CFA-treated rabbits.

Information obtained from the first year studies will be used as a basis for conducting the second and third year investigations.

We anticipate that some optimal time will be found when all CFA-treated rabbits will show definite but not excessive alveolar wall destruction. This time, possibly 12 weeks post CFA injection will be used as a point about which the smoking experiments will be run. For example, taking the

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12 weeks point, a group of 3 animals will receive whole cigarette smoke or gas-vapor phase from time zero (when CFA is injected) to 6 weeks post injection. A second group of 3 animals will be treated in the same way, except that they will inhale whole cigarette smoke or gas-vapor phase from the 9th through 15th weeks. A third group will be exposed from the 15th through the 21st weeks. "Control" rabbits, untreated with CFA, will be exposed to cigarette smoke in parallel, but not necessarily at the same time, as the CFA-treated rabbits. The number of weekly smoke exposures and duration of each exposure will have to be determined by pilot studies. Rabbits will be sacrificed at 24 weeks from the start of the experiment. The lungs will be handled as in the first year studies. Findings will be compared to the lungs of the first year project.

Third year studies are based on reports that whole cigarette smoke and gas-vapor phase influences the alveolar macrophages (1) and the lung microcirculation (2) respectively.

In order to determine the importance of the macrophage, and particularly its enzyme content, in the development of emphysema, compounds that delay or speed up the release of its cytoplasmic contents will be used. Their influence will be determined by modifications of the rate of development and severity of emphysema.

The animals will be given CFA as before. Beginning at approximately 7 days, when the macrophage response to the adjuvant is well developed, animals will be treated with stabilizers and labilizers and with stimulators and depressors of macrophage activity. Vitamin A (3, 4), carageenan (5) and streptolysin S (6) will be used as labilizers; chloroquine (7, 8) and hydrocortisone (9, 10) as stabilizers; glucan as a stimulator; and, methyl palmitate as a depressor (11). Appropriate controls will be used. Most of these agents, particularly in large quantities, are associated with multisystem side effects. The experiments will, of necessity, be controlled by paired animals--one receiving adjuvant plus added compound and the other only added compound. Gross and optical microscopic survey of the principal organ systems will be mandatory in these paired animal experiments.

Even if the accumulation of leukocytes and/or macrophages is important in the development of emphysema, their appearance is dependent on prior alterations of the microcirculation. There must be change in permeability, slowing of blood flow, margination and migration of white cells, platelet and local oxygen deficit. Therefore, alteration of the microcirculation should be investigated as an underlying cause of emphysema.

Incomplete Freund's adjuvant plus a capillary-damaging compound such as sodium tetradecyl sulfate will be used as a means of injuring the pulmonary microcirculation. The oil emulsion emboli reach this level of the circulation, persist for a short period, and should allow action by the capillary-damaging agent. Pilot studies will be run on a small

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number of animals using intravenous injection of adjuvant plus one of several trial levels of sodium tetradecyl sulfate. We are uncertain of the results of such an approach until tested and hesitate to generate a protocol beyond that listed for the study of emphysema above.

References:

1. Pratt, S. A., Smith, M. H., Ladman, A. J., and Finley, T. N. The ultrastructure of alveolar macrophages from human cigarette smokers and nonsmokers. *Lab. Invest.* 24: 331, 1971.
2. Williams, J. B., and Anderson, W. H. Acute effects of cigarette smoke on pulmonary ventilation-perfusion relationship. *Amer. Rev. Resp. Dis.* 98: 145, 1968.
3. Weissman, G. Labilization and stabilization of lysosomes. *Fed. Proc.* 23: 1038, 1964.
4. Shamberger, R. J. Inhibitory effect of vitamin A on carcinogenesis. *J. Nat. Cancer Inst.* 47: 667, 1971.
5. Cantanzaro, P. J., Schwartz, H. J., and Graham, R. C. Spectrum and possible mechanism of carrageenan cytotoxicity. *Amer. J. Path.* 64: 387, 1971.
6. Weissman, G., Becker, B., Wiedermann, G., and Bernheimer, A. W. Studies on lysosomes. VII. Acute and chronic arthritis produced by intra-articular injections of streptolysis S in rabbits. *Amer. J. Path.* 46: 129, 1965.
7. Abraham, R., and Hendy, R. Effects of chronic chloroquine treatment on lysosomes of rat liver cells. *Exp. Molec. Path.* 12: 148, 1970.
8. Read, W. K., and Bay, W. W. Basic cellular lesion in chloroquine toxicity. *Lab. Invest.* 24: 246, 1971.
9. Deodhar, S. D., and Bhaqwat, A. G. Desquamative interstitial pneumonia-like syndrome in rabbits. *Arch. Path.* 84: 54, 1967.
10. Merkow, L., Pardo, M., Epstein, S. M., Verney, E., and Sidransky, H. Lysosomal stability during phagocytosis of *Aspergillus flavus* spores by alveolar macrophages of cortisone treated mice. *Science* 160: 79, 1968.
11. Lentz, P. E., and DeLuzio, N. R. Biochemical characterization of Kupffer and parenchymal cells isolated from rat liver. *Exp. Cell Res.* 67: 17, 1971.

Illustrations given in Appendix, page 11.

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9. PHYSICAL FACILITIES AVAILABLE

General facilities available are those of the Department of Pathology and the School of Medicine of the University of Oregon.

A fully equipped electron microscope laboratory is in the Department of Pathology. This laboratory has an RCA EMU-3G and a Philips EM 200 electron microscope. Each electron microscope is housed separately and each has its own attached darkroom for developing negatives. The laboratory also contains a darkroom with all necessary equipment for enlarging the negatives to make final prints. In addition, there are present four automatic microtomes for producing sections for electron microscopy, a mechanical glass knifemaker, a vacuum evaporator, and a Smith-Farquhar tissue chopper.

General purpose laboratories are available in new departmental facilities.

Housing for small animals is adjacent to the pathology laboratories and a veterinarian is in attendance in the animal care facility.

11. BIOGRAPHICAL SKETCHES

a. Name; Title; Birthdate:

Robert E. Brooks, Ph.D.
Associate Professor of Pathology

REDACTED

b. Role in project:

Principal investigator.

c. Education; honors:

University of California, Los Angeles (physics)
University of Oregon Medical School, REDACTED athology)
University of Oregon Medical School, (pathology)
Recipient of a Special Fellowship from the National Cancer Institute,
1965-66.

d. Professional experience:

1972-present Associate Professor of Pathology, University of Oregon
Medical School.

1967-1972 Assistant Professor of Pathology, University of Oregon
Medical School.

1003539466

1961-1967 Instructor (electron microscopy), University of Oregon Medical School.
 1950-1961 Laboratory Technician, Department of Pathology, University of California Medical School, San Francisco.
 1948-1950 Laboratory Technician, Atomic Energy Section, University of California at Los Angeles.

a. Name; Title; Birthdate:

Richard D. Moore, M. D.
 Professor and Chairman, Department of Pathology
 REDACTED

b. Role in project:

Co-investigator.

c. Education; honors:

Gonzaga University, Spokane, Washington.
 Western Reserve University Medical School, Cleveland, Ohio, M. D., 1947.
 Sigma Xi

d. Professional experience:

1969-present Professor and Chairman, Department of Pathology, University of Oregon Medical School.
 1967-1969 Professor of Pathology, Case Western Reserve University, Cleveland, Ohio.
 1961-1969 Associate Pathologist, University Hospitals, Cleveland, Ohio.
 1957-1967 Associate Professor of Pathology, Western Reserve University.
 1956-1957 Assistant Professor of Pathology, University of Rochester, Rochester, New York.
 1955-1956 Assistant Professor of Pathology, Western Reserve University.
 1954-1956 Assistant Pathologist, University Hospitals, Cleveland.
 1953-1955 Senior Instructor in Pathology, Western Reserve University.
 1952-1953 Instructor in Pathology, Western Reserve University.

12. LIST OF PUBLICATIONS

a. Robert E. Brooks:

Brooks, R. E. Lung alveolar cell cytosomes: A consideration of their significance. Zeit. Zellforsch. 106: 484-497, 1970.

Brooks, R. E. Ultrastructural evidence for a noncellular lining layer of lung alveoli: A critical review. Arch. Intern. Med. 127: 426-428, 1971.

Brooks, R. E., and A. L. Tison, B. Intracellular components of neoplastic and normal alveolar cells from mouse lungs: Quantitative ultrastructural comparison. J. Nat. Cancer Inst. 47: 639-644, 1971.

1003539467

Brooks, R. E. Lung surfactant: An alternate hypothesis. Amer. Rev. Resp. Dis. 104: 585-586, 1971.

Brooks, R. E. Ultrastructure of lung lesions produced by ingested chemicals. I. Effect of the herbicide paraquat on mouse lung. Lab. Invest. 25: 536-545, 1971.

b. Richard D. Moore:

Moore, R. D., Lamm, M. E., Lockman, L., and Schoenberg, M. D. Cellular aspects of the action of Freund's adjuvant in the spleen and lymph nodes. Brit. J. Exp. Path. 44: 300-311, 1963.

Moore, R. D., and Schoenberg, M. D. Alveolar lining cells and pulmonary reticuloendothelial system of the rabbit. Amer. J. Path. 45: 991, 1006, 1964.

Moore, R. D., and Schoenberg, M. D. The response of the histiocytes and macrophages in the lungs of rabbits injected with Freund's adjuvant. Brit. J. Exp. Path. 45: 488-497, 1964.

Schoenberg, M. D., Stavitsky, A. B., Moore, R. D., and Freeman, M. J. Cellular sites of synthesis of rabbit immunoglobulins during primary response to diphtheria toxoid-Freund's adjuvant. J. Exp. Med. 121: 577-590, 1965.

Moore, R. D., and Schoenberg, M. D. A comparison of the primary and secondary response to complete Freund's adjuvant. Brit. J. Exp. Path. 47: 60-69, 1966.

1003539468

APPENDIX

Illustrations

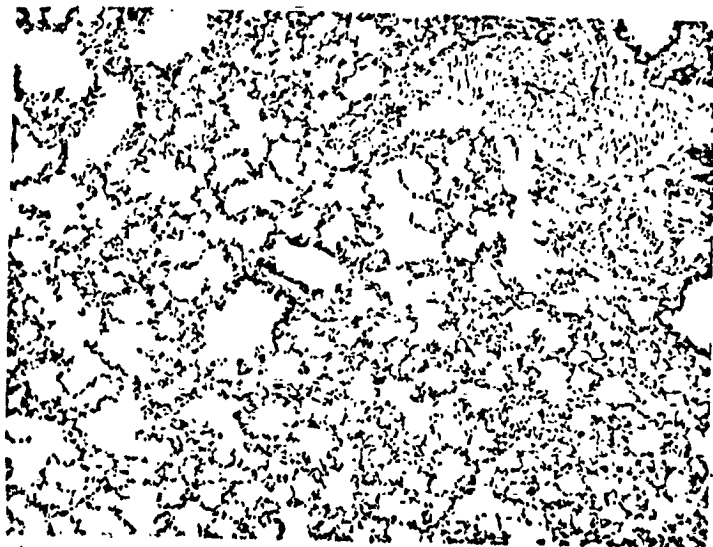
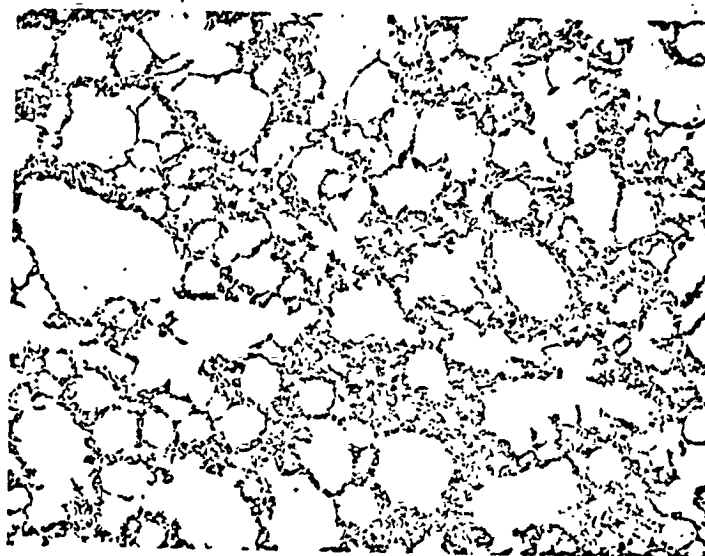
The figures are light micrographs taken of histological sections from uninflated rabbit lungs. Treated animals received a single intravenous injection, by ear vein, of 1.0 ml complete Freund's adjuvant. All micrographs are the same magnification, 72 X.

Figure Legends

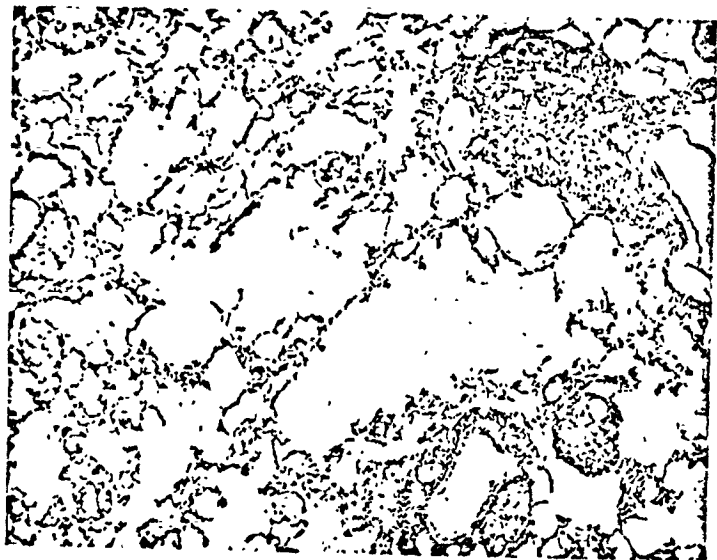
- Fig. 1. Untreated animal.
- Fig. 2. Two weeks after receiving adjuvant there are numerous collections of macrophages within air spaces and histiocytes in the walls. Distinct granulomas are present with a central collection of epithelioid cells and collar of lymphocytes.
- Fig. 3. Four weeks after receiving adjuvant, the inflammatory changes are similar to those seen at two weeks. The granulomas dominate the inflammatory reaction at this time.
- Fig. 4. Eight weeks after receiving adjuvant much of the exudate has been cleared from the lung. Many of the terminal air spaces are distended. This is more apparent when compared with Fig. 1.
- Fig. 5. Untreated animal.
- Fig. 6. This photomicrograph is also from an animal eight weeks after adjuvant. It is similar to Fig. 4, but illustrates two residual granulomas.
- Figs. 7 & 8. These illustrations are from animals twelve weeks after receiving adjuvant. There is still some residual inflammatory exudate in Fig. 7 and a small granuloma in Fig. 8. The distention of the terminal air spaces persists. Compare with Fig. 5 which is an illustration of the lung from an untreated animal.

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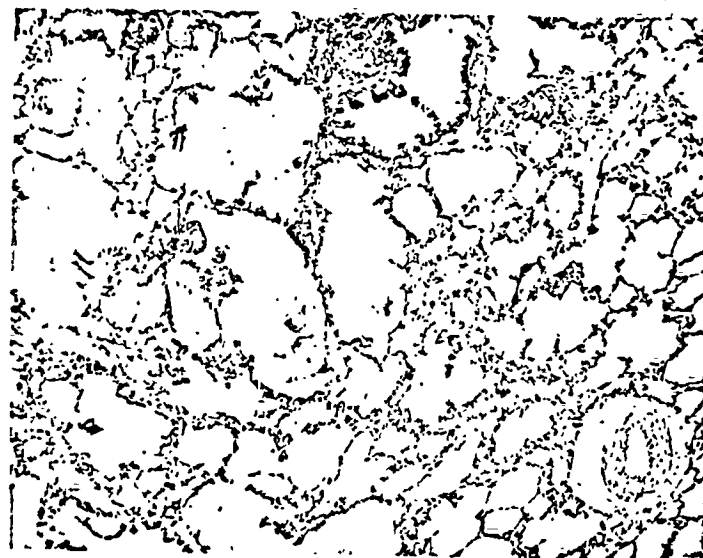
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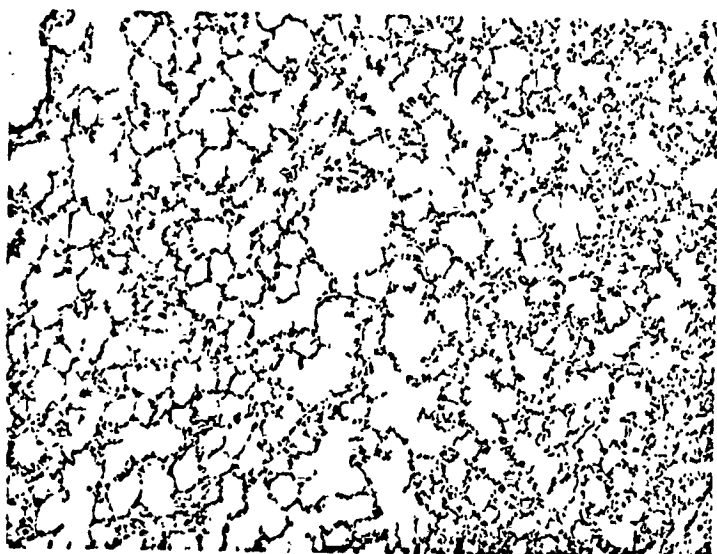
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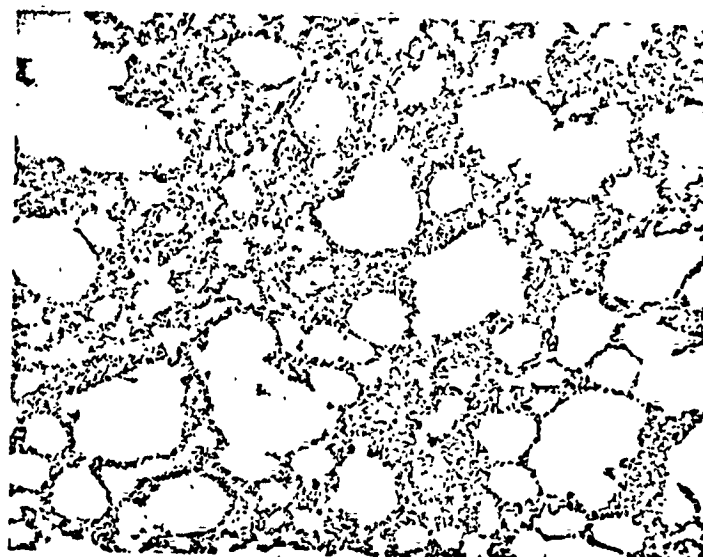
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#937 CROSS

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

August 8, 1973

Grant Application No. 937
PULMONARY

To: The committee comprising Drs. Gardner, Jacobson, Sommers,
and Wyatt

Subject: Carroll E. Cross, M.D., University of California, Davis
New application No. 937
"Cigarette Smoke Effects on Certain Aspects of Rat Lung
Metabolism"

History

An application from Dr. Cross was denied in 1969.

Grant No. 734, beginning in 1970, provided two renewals
and a supplement to support an originally approved three year
program.

Application No. 937 requests \$44,650 plus two additional
years. It competes as a new request both because there is no
commitment and because there is some reorientation of the program.

Documents Submitted

Attached is application dated July 30, 1973.

Also attached are C.V.'s of Drs. Cross, Goldstein, Mustafa,
and Chow.

Selected reprints and manuscripts of papers listed as
numbers 13, 14 and 15 on page 2f of the application have been
provided, and will be forwarded as you request.

Comment

Appended is a copy of Dr. Gardner's memorandum on his
July 9, 1973 visit to this applicant.

Following Dr. Cross's tradition, this application was
received late, but nevertheless was accepted.


F.W.N.

FWN:wg
Encls.

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Comm.

Dr. Gardner
Dr. Jacobson
Dr. Sommers
Dr. Wyatt

PULMONARY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8985

AUG 8 1973

Application for Research Grant

(Use extra pages as needed)

Date:

July 30, 1973

1. Principal Investigator (give title and degrees):

Carroll E. Cross, M.D., Associate Professor and Director, Section of Pulmonary Medicine
Co-investigators: Elliot Goldstein, M.D., Assoc. Prof., Section of Infectious Diseases
Ching Chow, Ph.D., Assistant Research Biochemist, U.C. Davis Pulmonary Research Lab.
Mohammad Mustafa, Ph.D., Assistant Research Biochemist, U.C. Davis Pulmonary Res. Lab.

2. Institution & address:

Regents of the University of California
University of California at Davis
Davis, California 95616

3. Department(s) where research will be done or collaboration provided:

Department of Internal Medicine (Drs. Cross and Goldstein)
Department of Biological Chemistry (Dr. Mustafa)
Primate Center Research Facility (Drs. Mustafa and Chow)

4. Short title of study:

Cigarette Smoke Effects on Certain Aspects of Rat Lung Metabolism

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 30 months.

7. Brief description of specific research aims:

The proposed studies are intended to accomplish the following specific research aims:

i) to determine the effects of long-term cigarette smoking on rat lung enzymes related to the pentose shunt and to glutathione metabolism; on rat lung lysosomal enzymes; on rat lung alveolar macrophages; and on rat anti-bacterial defense mechanisms.

ii) to determine the effects of long-term cigarette smoking minus particulates on the above mentioned lung biochemical and antimicrobial parameters

iii) to determine the interacting effects of long-term cigarette smoking and oxidant pollutant (ozone) exposure on the above mentioned lung biochemical and antimicrobial parameters.

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Preliminary studies in our laboratory have demonstrated that experimental rat exposures to cigarette smoke four times daily for five weeks result in significant increases in lung glucose-6-phosphate dehydrogenase (a key enzyme in the pentose shunt that subserves generation of "reducing equivalents") and increases the number of resident alveolar macrophages. It is thus our working hypothesis that long-term cigarette smoking does effect lung parenchymal metabolic processes, that these effects result in augmentation of certain enzymes related to pentose shunt activity, and that this could conceivably result in an augmented lung antioxidant defense capability. We intend to test this hypothesis by determining changes induced by cigarette smoking on the activities of a number of enzymes subserving lung glutathione metabolism and the superimposed effects of cigarette smoking and oxidants on these systems. We intend to determine if the particulate phase contributes to this metabolic adaptation by looking at the effects of both whole cigarette smoke and cigarette smoke without particulates on the forementioned lung metabolic parameters.

Since increases in resident alveolar macrophages have been reported to occur in human smokers we also propose to look at the number of alveolar macrophages that can be obtained by alveolar lavage in cigarette smoking rats and attempt to correlate this with particulate inhalation and with a simultaneous assessment of lung antibacterial defense systems.

Since we have found that high dose short-term oxidant exposures compromise lung antioxidant defense systems whereas low dose long-term oxidant exposures augment lung antioxidant defense systems, we plan to examine the effects of prior cigarette smoke exposure on lung short-term and long-term oxidant susceptibility.

Finally, since cigarette smoking causes an increase in lung alveolar macrophages in humans and since these cells have a high content of potentially damaging lysosomal enzymes, we plan to assess potential cigarette-induced lability (or intrapulmonary release) of these lysosomal enzymes by assessing both particulate phase and cytosol phase lysosomal enzyme activity in lungs exposed to cigarette smoke.

9. Details of experimental design and procedures (append extra pages as necessary)

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1. Introduction

The lung is an organ at risk from the effects of noxious inhaled environmental agents. As such, the lung has been provided with a number of self-protective defense systems. This study is designed to study the effects of cigarette smoking on two of these defense systems, mainly lung antioxidant defenses and lung antibacterial defenses. In addition, the study will include assessments of the interacting effects of cigarette smoking and exposure to oxidant pollutants and assessments of lung lysosomal enzyme systems.

It has been shown recently that sulfhydryl-containing compounds are susceptible to such oxidant stresses as ionizing radiation (1), high oxygen tension (2), and ozone (3-5). These compounds, which are primarily glutathione and cysteine, have been postulated to be part of an antioxidant defense system in lung cells (6). Preliminary studies in this laboratory have suggested that although chronic cigarette exposure does not particularly affect the total amount of sulfhydryl-containing compounds in the lung, this exposure does alter the ratio of reduced to oxidized compounds in the cell (see III B). This alteration has a potential effect on the ability of the lung to withstand oxidant stress. For this reason, this study proposes to systematically characterize cigarette smoke-induced changes in lung glutathione and cysteine concentrations. This characterization will include chromatographic analysis of sulfur-containing compounds and spectrophotometric analysis of these compounds.

Previous reports have suggested that pretreatment with certain compounds give a measure of protection from subsequent oxidant challenge. For example, alpha-naphthylthiourea induces tolerance to high oxygen tension (8). Sublethal doses of ozone protect an animal from challenging doses of ozone (9). Considering the fact that cigarette smoke contains measurable amounts of over 250 different compounds, including a number that can be considered oxidants (10), it is possible that preexposure of animals to cigarette smoke can affect the animals subsequent susceptibility to oxidant stress.

Chow and Tappel have proposed an enzymatic mechanism for disposing of lipid peroxides generated by oxidants (11). There exists some data to support this mechanism (12,13). Cigarette smoke has been shown to alter certain enzyme activities. Most notably studied has been benzpyrene hydroxylase (14-17). Although there is considerable data that whereas the effects of whole cigarette smoke and volatile phase only (particulates removed) have been elucidated with regard to the ciliostatic effects of cigarette smoke and with regards to the pulmonary alveolar macrophage antibacterial defense system of the lung, there has been little study of the differential effects of whole smoke as compared to volatile phase on lung tissue biochemical responses to cigarette smoke.

Our preliminary data shows a cigarette smoke-induced augmentation of the activity of glucose-6-phosphate dehydrogenase in lung tissue (see III C). This suggests that cigarette smoke could potentially augment other enzymes related to the pentose cycle and to antioxidant defense systems. In the current proposal we plan to study further this aspect of cigarette smoke effects on the lung and to elucidate further the part played by particulates and by volatile phase on this response.

Recent studies performed by a colleague of ours at U.C. Davis have shown that ozone exposure increases lysosomal enzyme activities (18). Observations from other laboratories have indicated that cigarette smoke exposure increases lysosomal enzyme activities in pulmonary macrophages (19). Since increased activities of proteolytic lysosomal enzymes have been implicated in the

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pathogenesis of pulmonary diseases (20), especially in hereditary α_1 -antitrypsin deficiency (21,22), it is important to establish whether there is any additive effect of combined cigarette smoke exposure and ozone exposure on lung lysosomal enzyme activities. This project intends to study the interaction of cigarette smoke exposure and ozone exposure as seen in lung lysosomal enzyme systems.

One of the thrusts of the current proposal is to study the combined interacting effects of cigarette smoke exposure and ozone exposure on aspects of lung pathophysiology. This is apparent from comments made above concerning the combined effects of cigarette smoke and ozone exposure on lung antioxidant defense systems and on lung lysosomal enzyme systems. Two further examples can perhaps be mentioned here.

It is known that ozone exposure produces pulmonary edema, with the extent of pulmonary edema determined by dose (23,24). Cigarette smoking is not known to induce pulmonary edema. However, exposure to cigarette smoke might inhibit or potentiate ozone-induced pulmonary edema. This project intends to study ozone-induced pulmonary edema and any effect that cigarette smoke might have on this edema. Of particular interest is the very low-grade edema associated with relatively low doses of ozone. Since these relatively low doses of ozone are found in the atmosphere of many major cities, it is of interest to examine the interacting effects of cigarette smoke exposure and low-dose ozone exposure on low-grade pulmonary edema. This edema can be sensitively measured by the method of Alpert et al (25), involving the measurement of lung accumulation of radioactively labelled protein.

Finally, it has been shown that ozone exposure decreases pulmonary bacterial clearance (26). Furthermore, cigarette smoke exposure is known to decrease pulmonary bacterial clearance (27). It is of interest to determine whether there are any additive effects of cigarette smoke exposure and ozone exposure on pulmonary bacterial clearance.

II. Methods and Procedure

Subjects will be 30-day old male Sprague-Dawley rats which are free of chronic respiratory diseases. In general, the 30 day old rats (or alternatively 60 day old rats) will be exposed to cigarette smoke for 5-10 week long periods after which they will be subsequently or concomitantly exposed to room air or oxidant pollutants. At the conclusion of a predetermined exposure period a variety of studies concerned with lung metabolism will be done.

A. Cigarette exposures. These will be carried out in a Walton Horizontal Cigarette Smoking Machine (or alternative model). Initial studies will be accomplished on the combined volatile and particulate phase of cigarette smoke whereas a second series of studies will involve biological assessment of the volatile phase only, the particulate phase being filtered out by the Cambridge Filter. Exposures will be for 4 times daily, 10 minutes each, for 5-10 weeks utilizing the IRI 1A3 alkaloid series reference cigarette under standardized conditions of flow, volume and temperature.

For rat exposures the rats will be divided up into groups similar to groupings shown in III B. Some of the groups will, subsequent to cigarette smoke exposure, receive exposure to oxidant pollutants.

B. Ozone exposures. Ozone exposures will be carried out in specially designed chambers at the U.C.D. Air Pollution Exposure Facility. Ozone is

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generated by passing oxygen thru an electric silent arc ozone generator (28). The ozone is conveyed to the chambers at a flow of 100 liters per minute. The closed-system chambers have a volume of (27 cu ft). Air flow through the chambers is such that there are 10 changes of air per minute. These chambers are equipped for any range of ozone exposure from 0.1 ppm to 10 ppm. The temperature is maintained at 75° and the relative humidity is maintained at 50%.

C. Antibacterial defenses. Pulmonary bacterial clearance will be measured according to the method described in Goldstein et al. (29). Briefly, this method consists of offering the animals an aerosol of radiolabeled microorganisms. After a suitable incubation period, the animals are sacrificed and the lungs are homogenized. The homogenate is assayed for the radiolabel, which is a function of the number of bacterial cell bodies remaining in the lungs. The lung homogenate is also cultured for visible bacteria, to assess pulmonary bacterial clearance. These two determinations give an indication of the number of organisms cleared from the lung per unit time, and also the number of organisms killed per unit time. This method produces an assessment of the extent of pulmonary anti-bacterial defense mechanisms.

D. Enzyme determinations. At the conclusion of the exposures rats will be weighed, anesthetized with pentobarbital and decapitated. Each set of lungs will be perfused with cold saline, and homogenized in isotonic sucrose-mannitol-Tris buffer with a glass-Teflon homogenizer. The homogenate will be filtered through cheesecloth and brought to 13 ml total volume by addition of buffer. The homogenate will then be apportioned for the particular assays to be done.

Glucose-6-phosphate dehydrogenase will be assayed according to the method of Lohr and Waller (30). Glutathione reductase will be assayed according to the method of Horn (31). Glutathione peroxidase will be measured according to the method described by Little and O'Brien (32). Protein content will be measured by the Lowrey method (33). In some experiments we will also determine overall comparative lung protein, RNA and DNA synthesis rates as determined by standard isotope incorporation rates.

Four specific proteolytic lysosomal enzymes will be studied. The peptidases cathepsin A, B, and C and the protease cathepsin D will be measured. The assay will be performed according to the method described by Misaka and Tappel (34). Three additional lysosomal enzymes will be studied. Lysozyme, acid phosphatase, and β -N-acetyl-glucosaminidase all appear to have relatively high specific activity in lung tissue (35). Lysozyme will be measured by a standard microbial bioassay technique (36). Acid phosphatase will be measured by the method of DeDuve et al (37). β -N-acetyl-glucosaminidase will be assayed according to the method of Beck (38).

E. Morphological correlations. It is possible that exposure to cigarette smoke would induce a hyperplasia of the bronchial lining membranes and an increase in free macrophages in the regions of the terminal bronchioles and alveoli, as has been described in rodents following exposure to smog (39). As this could influence the biochemical observations being made, and in order to interpret the biochemical observations being made, it will be necessary to have morphological studies done in conjunction with the above outlined studies. Representative lungs from all the rat groups will thus be obtained for light microscopic and E.M. study under the direction of our University of California colleagues,

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Drs. Walter Tyler and Donald Dungworth of the U.C. Davis School of Veterinary Medicine. They are already doing extensive rat histological and E.M. studies as a part of our combined ozone exposure effects on rodent lung studies (40,41). We plan to attempt to correlate these anatomical sections with the number of free macrophages obtained by lung lavage, with lung DNA and protein synthesis rates as determined by isotope incorporation techniques and with the enzyme measurements discussed in D above.

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III. Prior Related Work

A. Publications Supported by the Council for Tobacco Research, U.S.A., 1970-73

C.E. Cross, U.C. Davis School of Medicine, Principal Investigator

1. Mustafa, M.G., and Cross, C.E.: Localization of $\text{Na}^+\text{-K}^+$, Mg^{++} ATPase in alveolar macrophage subcellular fractions. *Life Sci.*, 9: 947-54, 1970.
2. Mustafa, M.G., Cross, C.E., Tyler, W.S., and Duffy, M.J.: Interference of cadmium ion with oxidative metabolism of alveolar macrophages. *Arch. Int. Med.* 127: 1069-77, 1971.
3. Brunstetter, M.A., Hardie, J.A., Schiff, R., Lewis, J.P., and Cross, C.E.: The origin of pulmonary alveolar macrophages. *Arch. Int. Med.* 127: 1064-68, 1971.
4. Cross, C.E., Mustafa, M.G., Peterson, P., and Hardie, J.A.: Pulmonary alveolar macrophage: Membrane associated Na^+ , K^+ , Mg^{++} -Adenosine triphosphatase system. *Arch. Int. Med.* 127: 1069-77, 1971.
5. Mustafa, M.G., Peterson, P.A., Munn, R.J., and Cross, C.E.: Effects of Cd^{++} on metabolism of lung cells. *Proceedings of the Second International Clear Air Congress*, Edited by H.M. Englund and W.T. Berry, Academic Press, N.Y., p. 143-151, 1971.
6. Cross, C.E., Ibrahim, A.B., Ahmed, M., and Mustafa, M.G.: Effects of Cd^{++} on respiration and ATPase activity of the pulmonary alveolar macrophage: A model for the study of environmental interference with pulmonary cell function. *Environ. Res.* 3: 512-20, 1970.
7. Mustafa, M.G., Cross, C.E., Munn, R.J., and Hardie, J.A.: Effects of divalent metal ions on alveolar macrophage membrane adenosine triphosphatase activity. *J. Lab. Clin. Med.* 76: 563-71, 1971.
8. Cross, C.E., Le, C.T., and Mustafa, M.G.: Effects of ozone on some pulmonary alveolar macrophage (PAM) biochemical reactions. *Clin. Res.* 19: 147, 1971 (abstract).
9. Mustafa, M.G., Cronin, S.R., Duvall, T.R., and Cross, C.E.: Ozone effect on activities of lung cellular membrane enzymes. *Clin. Res.* 20: 197, 1972 (abstract).
10. DeLucia, A.J., Hoque, P.M., Cross, C.E., and Mustafa, M.G.: Sulfhydryl content of rodent lungs under oxidant stress. *Clin. Res.* 20: 240, 1972.

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11. DeLucia, A.J., Hoque, P.M., Mustafa, M.G., and Cross, C.E.: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. *J. Lab. Clin. Med.* 80: 559-66, 1972.
12. DeLucia, A.J., Mustafa, M.G., and Cross, C.E.: Glutathione and sulfhydryl-containing enzyme activities in lung under ozone stress. *Clin. Res.* 21: 223, 1973 (abstract).
- ✓ 13. York, G.K., Arth, C., Stumbo, J.A., Cross, C.E., and Mustafa, M.G.: Effect of cigarette smoke and tobacco extract on lung macrophage function. *Arch. Environ. Health*, in press, 1973.
- ✓ 14. Mustafa, M.G., DeLucia, A.J., York, G.K., Arth, C., and Cross, C.E.: Ozone interaction with rodent lung: Effects on oxygen consumption of mitochondria. *J. Lab. Clin. Med.*, in press, 1973.
- ✓ 15. Mustafa, M.G., DeLucia, A.J., Cross, C.E., York, G.K., and Dungworth, D.C.: Effect of ozone exposure on lung mitochondrial oxidative metabolism. *Chest*, in press, 1974.
16. York, G.K., Cross, C.E., Mustafa, M.G., and Bodai, B.I.: Lung glucose-6-phosphate dehydrogenase stimulation by cigarette smoke. Effects of short-term ozone exposure. Submitted for publication, 1973.
17. Mustafa, M.G., and Cross, C.E.: Effects of short-term ozone exposure on lung mitochondrial oxidative and energy metabolism. Submitted for publication, 1973.
18. Mustafa, M.G., and Cross, C.E.: Lung cell mitochondria: Rapid oxidation of glycerol-1-phosphate but slow oxidation of 3-hydroxybutyrate. Submitted for publication, 1973.

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B. Preliminary Protocol January-June 1973,...A study of Cigarette smoke effects on selected aspects of lung metabolism.

i) Aim: To accomplish a preliminary study of the effects of cigarette smoke and cigarette smoke plus oxidants on selected aspects of lung metabolism.

ii) Introduction: It has been shown experimentally that chronic cigarette smoking influences lung biochemistry both in vivo and in vitro (42-48). It has also been shown that oxidant pollutants interact with a number of important lung biochemical reactions (49-51) including lung antibacterial defense systems (52). This protocol is intended to gather preliminary data on the effects of cigarette smoking and on the effects of cigarette smoking plus oxidant pollutant exposure on a number of lung enzyme systems known to be influenced by both high dose short-term and low dose long-term exposures to ozone. Since certain constituents of cigarette smoking represent oxidants (53) and since both cigarette smoking (43,44) and oxidant pollutants (51) are known to induce certain lung enzymes, this would seem a priori a reasonable hypothesis to test. One interesting question is whether cigarette smoke is capable of inducing enzymes which are proposed to be responsible for oxidant protection, i.e. glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase (51).

Another interesting question relates to the combined effects of cigarette smoke and oxidants on the "redox state" of lung tissue. Parameters suggested for study include lung glutathione levels and ratios of reduced to oxidized reactive lung thiol groups. Of course, it will be necessary to make concomitant histological observations in order to interpret the biochemical data being generated.

iii) Methods:

a. Cigarette smoke exposures

Thirty two male Sprague-Dawley CRD-free rats, aged 30 days, are the subjects of the experiment. These rats are to be weighed and divided into four groups of eight rats, as follows:

<u>Group</u>	<u>Exposure Conditions</u>
A	sham cigarette exposure, sham O ₃ exposure (control group)
B	sham cigarette exposure O ₃ 3.0 ppm x 4 hr.
C	cigarette exposure 4/day x 35 days, sham O ₃ exposure
D	cigarette exposure 4/day x 35 days, O ₃ 3.0 ppm x 4 hr.

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Half of the rats are to be exposed to cigarette smoke 4 times per day for 35 consecutive days. Each exposure consists of 9 puffs of a 85 mm non-filtered American cigarette. Reference IRI 1A3 cigarettes are to be used in the future. Each puff consists of 30 ml of smoke in a 378 ml chamber (i.e. 1:12 dilution). A Walton Horizontal Cigarette Exposure Machine is to be used for the exposures. The sham-exposed rats are to be placed in the chambers and given 9 puffs of air in a ten-minute period four times daily.

b. Ozone exposures

After 35 consecutive days of smoke exposure, two groups (B and D) of the rats will be subjected to 3.0 ppm O₃ for four hours. Ozone is to be delivered under standard conditions of flow and temperature at the Air Pollution Exposure Laboratory at U.C.Davis.

c. Experimental

Immediately after the ozone exposure, the rats are weighed, anesthetized with pentobarbital and decapitated. Blood is collected for complete blood counts. Lungs are to be perfused with cold saline and homogenized in isotonic sucrose-mannitol-Tris buffer with a glass-Teflon homogenizer. The homogenate is to be filtered through cheesecloth and brought to 16 ml total volume by addition of isotonic sucrose-mannitol-Tris buffer.

The following determinations will be made on the obtained lung tissue:

Homogenate Assays

- Reduced glutathione
- Oxidized glutathione
- Mitochondrial respiration
- Total sulfhydryls
- Non-protein sulfhydryls
- Oxidized non-protein sulfhydryls
- DNA determination
- Protein determination

Mitochondrial Assays

- NADH - cytochrome c reductase
- Succinate-cytochrome c reductase

Microsomal Assay

- NADPH - cytochrome c reductase

Cytosol Assays

- glucose-6-phosphate dehydrogenase

d. Critique of initial protocol

Several factors of interest were not evaluated in this preliminary experiment. First, no histology or electron microscopy studies were done. Second, the lungs were not evaluated for any changes in cell population; of

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particular interest would have been an estimation of the number of type II cells and/or alveolar macrophages in both the control and the cigarette exposure groups. Third, an increased number of blood assays could have been performed, including evaluation of hematopoiesis. Fourth, the anti-oxidant enzyme systems of glutathione reductase - glutathione peroxidase were not evaluated in the preliminary experiment. Fifth, no determination was made of lung lysosomal enzyme systems, both total and compartmentalized, in the preliminary protocol. All of these defects will be remedied in future protocols.

iv) Results of first preliminary protocol

a. Meaning of groups

A = sham ozone exposure; sham cigarette exposure (control)

B = ozone-exposed; sham cigarette exposure

C = sham ozone exposure; cigarette exposed

D = ozone exposed and cigarette exposed

b. G-6-PD

In the G-6-PD assay, there was a significant difference between smokers and non-smokers (see Fig. 1). Group B was insignificantly elevated over Group A, but Group C was elevated 54% of control, and Group D was 32% of control. There is also a drop of 22% between Group C and Group D, suggesting the influence of ozone superimposed on the cigarette smoke effect on this enzyme.

c. Non-protein sulphydryls

In figure 2, we see the results of reduced and oxidized non-protein SH determinations. There is a 12% drop in total NPSH (i.e. reduced NPSH) between Group A and Group B. There is an even further drop of 21% in non-reduced NPSH from Group A to Group B. This is an expected drop since the ozonized Group B would be expected to lose some NPSH to higher oxidation status.

There is only a very slight drop between Group A and Group C; this could signify that chronic exposure to cigarette smoke does not appreciably reduce NPSH levels.

There is a 9% drop in reduced NPSH between Group D and Group A, and a 5% drop between Group D and Group C. There is a 7% rise in non-reduced NPSH between Group C and D.

d. Glutathione

The ratio of GSH/GSSG dropped 44% from Group A to Group B (see fig. 3). This represents a significant relative oxidation of glutathione after oxidation by ozone. This is an expected result of ozone exposure. There was a 30% rise in GSH/GSSG between Group A and Group C representing relative reduction by cigarette smoke. This was not expected, but it may help to explain certain other features of this experiment. There was little difference in GSH/GSSG between Group A and Group D; this perhaps signifies that there is oxidation by ozone, but that the extent of oxidation is less between A and B than between C and D.

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There was a 43% drop in GSH between Group A and Group B (see Fig. 4). This was expected. What was not expected was a 21% drop in GSH between Group A and Group C, and no drop at all between Group C and Group D. This perhaps means that there is some other NPSH which is responsible for the slight drop in NPSH between C and D.

e. White cell count

There was no significant difference in white cell count between Group A, Group B, and Group C, although there was some drop between A and B. There was a significant drop between Group A and Group D.

f. Other assays

The other assays done (i.e. reductases and respiration) yielded insignificant data. Platelet counts and hematocrits showed no difference between control and experimental groups.

v) Methods, second preliminary protocol

Exposure conditions will be identical to the first protocol. We will eliminate platelet counts and perhaps the reductase assays. We will add macrophage counts, alveolar lavage albumin assay, and GSH reductase - GSH peroxidase assay.

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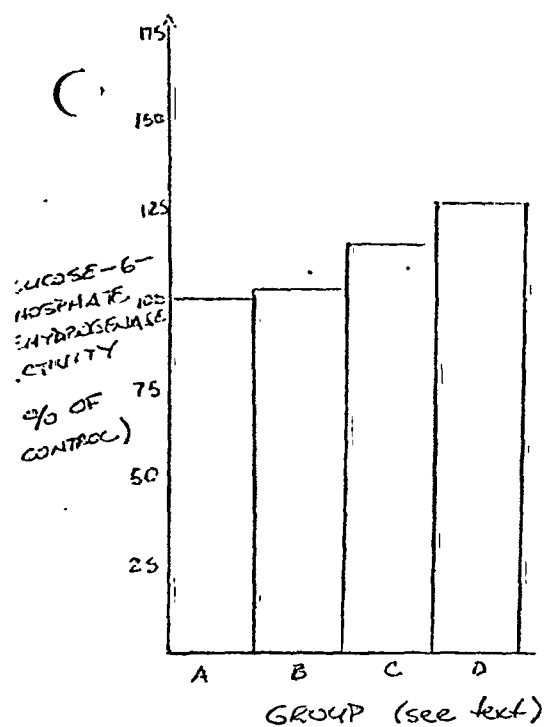


FIGURE 1.

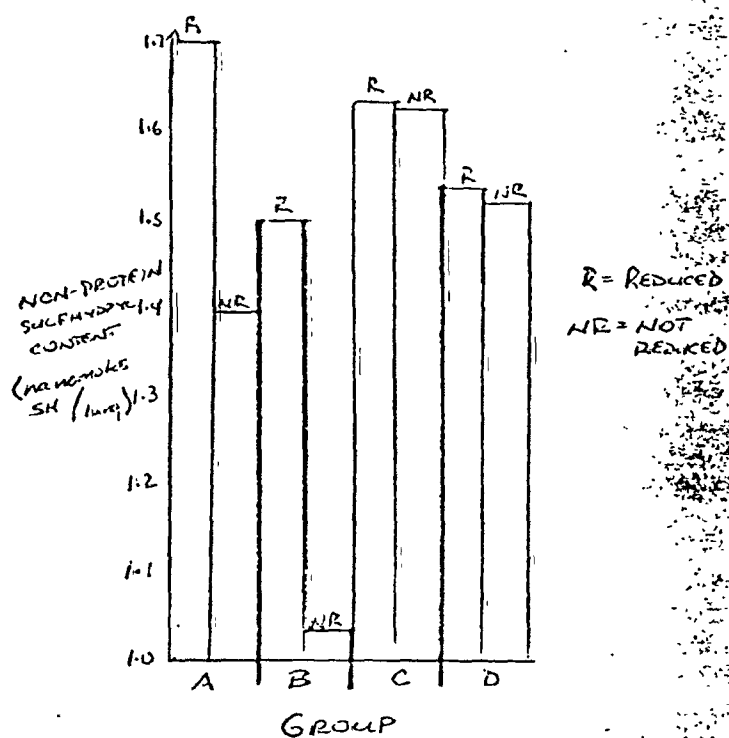


FIGURE 2.

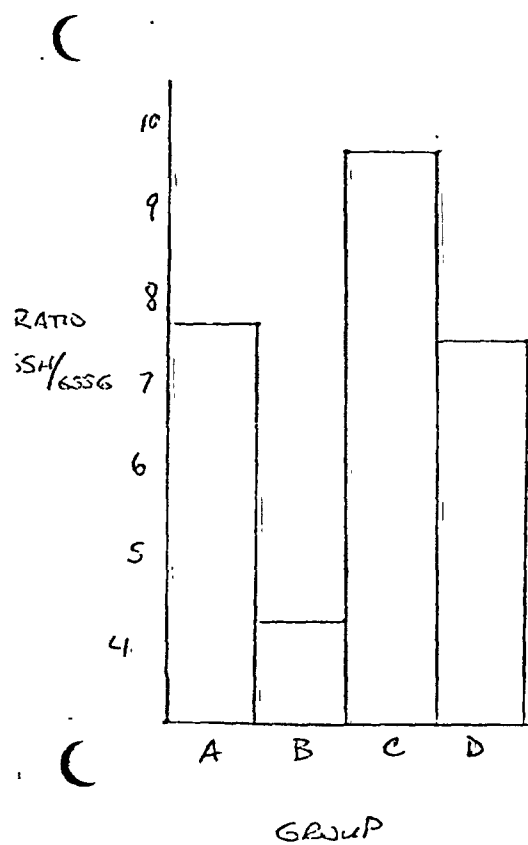


FIGURE 3.

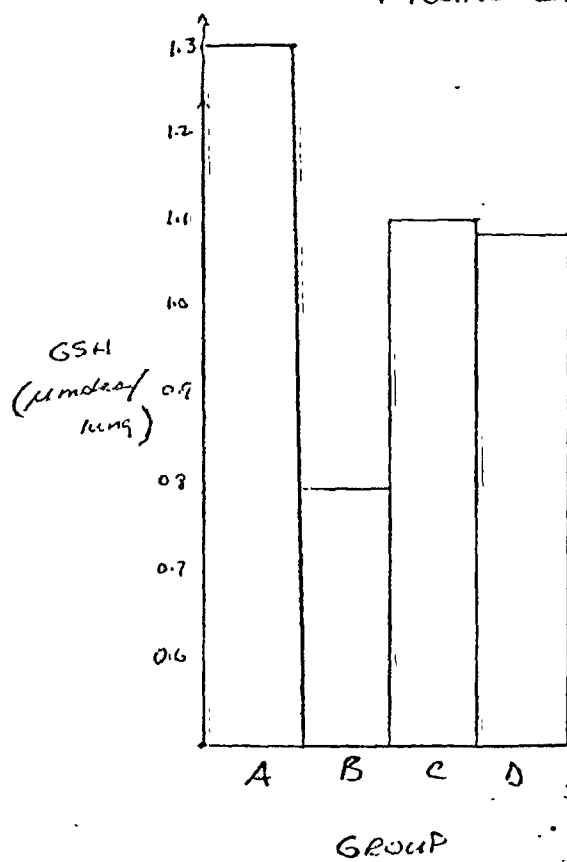


FIGURE 4.

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STIMULATION OF LUNG GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY BY CIGARETTE SMOKE.

EFFECTS OF SHORT-TERM OZONE EXPOSURE¹

Recently three groups of investigators have demonstrated significant increases in glucose-6-phosphate dehydrogenase (G-6-PD) activities in lung tissue following oxidant exposure²⁻⁵. The significance of the augmented G-6-PD activity most probably relates to increased hexose monophosphate shunt activity required by reparative processes in the lung, e.g., increased nucleotide production (for nucleic acid synthesis) and increased NADPH production (required for a number of oxido-reductive biosyntheses)⁶⁻⁸. However, the augmented G-6-PD activity might also speculatively relate to an increased supply of NADPH available for the reduction of oxidized glutathione (GSSG), a mechanism operative in red cells and which may presumably relate to lung protective anti-oxidant defense capabilities.

Cigarette smoke exposure has been reported to modify a number of lung enzyme activities, including those related to alveolar macrophage function⁹⁻¹¹ and to lung metabolism¹²⁻¹⁴. This report describes the effects of cigarette smoke exposure on lung G-6-PD activity. Since both exposure to cigarette smoke and to air pollutant have been associated with bronchopulmonary diseases, including an increased susceptibility to respiratory infections^{15,16}, the effects of a superimposed acute O₃ exposure were also determined.

Forty 30-day-old Sprague-Dawley rats (originally specific pathogen free) were divided into four groups. Ten rats served as control and received 35 days of sham cigarette smoke exposure plus 4 hours of sham O₃ exposure; ten received 35 days of sham cigarette smoke exposure followed by 4 hours of exposure to 3 ppm O₃; ten received 35 days of cigarette smoke exposure followed by sham O₃ exposure; and ten received 35 days

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of cigarette smoke exposure followed by 4 hours of exposure to 3 ppm O₃.

The cigarette smoke exposures consisted of 9 puffs of cigarette smoke in a ten-minute period (Pall Mall 85 mm) 4 times daily for 5 weeks in a Walton Horizontal Cigarette Exposure Machine¹⁷ set to give 1:12 smoke:air ratio at a puff volume of 30 ml. Sham cigarette smoke exposures consisted of puffs of room air delivered through the same apparatus. Ozone exposures consisted of 3.0 ppm (\pm 0.1) ozone for 4 hours in a modified fiberglass environment unit described previously^{5,18}. Sham ozone exposures consisted of room air exposures in identical units.

Animals were anesthetized with pentobarbitol and exsanguinated. The lungs were perfused with cold isotonic saline via the pulmonary artery, then trimmed of excess bronchovascular and pleural connective tissue.

The lungs were then homogenized with a glass Teflon homogenizer, using an isotonic medium (0.15 M sucrose, 0.15 M mannitol, 1 mM Tris). The

homogenate was filtered through two layers of cheesecloth. The homogenate was then centrifuged at 600 x g for ten minutes to remove nuclei and cellular debris and subsequently at 37,000 x g for twenty minutes to remove mitochondria and microsomes. The supernatant (cytosol) was then used for enzyme assay.

G-6-PD activity in the cytosol was determined according to the method of Lohr and Waller¹⁹. Protein concentration was determined by the method of Lowry²⁰.

G-6-PD activities in lung cytosol from control, ozone exposed, cigarette exposed, and cigarette plus ozone exposed rats are shown in Table 1. Control rats showed an activity of 39.2 ± 9.1 nanomoles NADPH formed/min/mg protein at 23°C. Rats exposed to 3.0 ppm ozone for 4 hours showed a 12% decrease, similar to findings reported previously⁵. Rats exposed to cigarette

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smoke for 5 weeks showed a 28% increase relative to untreated control rats. In cigarette smoking rats subsequently exposed to ozone there was 7% increase over control rats but a decrease of 20% compared to the cigarette smoking group not exposed to ozone. Rats exposed to cigarette smoke and subsequently exposed to ozone showed a 21% increase over rats exposed to ozone only.

Lung G-6-PD activity has been the object of study by a number of investigators concerned with the injury-reparative processes in the lung, including that due to high tensions of oxygen², short-term ozone exposure⁵, long-term ozone exposure^{3,4} and silica dust inhalation²¹. Although G-6-PD activity represents only one enzymatic step in the hexose monophosphate shunt, it occupies a key position in overall glucose metabolism in the lung and has been used to estimate the activity of the hexose monophosphate pathway^{22,23}. The observation that chronic cigarette smoking stimulates lung G-6-PD activity suggests that cigarette smoking augments overall activity of the hexose monophosphate shunt in lung tissue. The observation that short-term, high level exposures to ozone inhibits lung G-6-PD activity verifies previous data indicating that this oxidant inhalant under these conditions rather non-discriminantly decreases the activity of a number of lung enzyme systems^{5,24}.

The present studies do not allow further conclusions to be drawn concerning the mechanisms involved in the cigarette smoking induced increases in G-6-PD activity. It is possible that processes such as cellular hyperplasia, cellular proliferation or increased cell renewal could account for the augmented activity⁶⁻⁸. In addition, the question arises as to which cells in the lung are primarily affected by the smoke exposure. In this context it is particularly interesting that cigarette smoking exposure increases the number of alveolar macrophages appearing in the lung^{25,26} and that these cells have been noted to possess an

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active pentose shunt metabolism²⁷. In addition, it is an attractive assumption that the augmented shunt activity, by producing NADPH, might be involved in determining lung susceptibility to oxidant stress much as red cell G-6-PD activity and related NADPH generation help to determine red cell antioxidant defense capabilities²⁸. This assumption will require further validation by determination of the activities of related antioxidant enzyme systems in the lung such as glutathione, glutathione reductase and glutathione peroxidase.

The composition of cigarette smoke is complex. Biological and biochemical testing of whole cigarette smoke should be conducted with this limitation in mind. The merit of the present biochemical assay of a parenchymal lung tissue response to whole cigarette smoke suggests that future studies with simpler extracts of cigarette smoke, such as with the particulate phase removed, would be a worthwhile undertaking.

In summary, the results demonstrate that experimental cigarette smoking in rodents for 35 days increases lung G-6-PD activity. Short-term exposures to high levels of ozone (3 ppm, 4 hrs.) decreases lung G-6-PD activity whereas combined cigarette smoke and short-term high level O₃ exposures give an intermediate effect. The neutralization of opposing effects of ozone and cigarette exposure, so that rodents exposed to both agents show little difference from control animals, suggests that there may be a diminished susceptibility of the smokers to oxidant exposure. The importance of this lung enzymatic effect with respect to overall lung function of the smoker is unknown.

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Table 1. Effect of Long-Term Cigarette Smoke Exposure and Short-Term Ozone Exposure on Glucose-6-Phosphate Dehydrogenase Activity in Rat Lung Cytosol

Group	A	B	C	D
Exposure Conditions	Control	Ozone	Cig. Smoke	Cig. Smoke + O ₃
No. of Rats	10	10	10	10
G-6-PD Activity (nanomoles NADPH formed/ min/mg protein)	39.19	34.79	50.16	42.10
	<u>+9.1</u>	<u>+12.8</u>	<u>+16.3</u>	<u>+18.6</u>
% of Control	-	88%	127%	107%
P vs control (A)	-	<.2	<.05	n.s.
P vs ozone (B)	-	-	<.025	<.2
P vs cigarette (C)	-	-	-	<.2

Statistical analysis by Student's T test. n.s. = not significant

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IV. Significance

Although the effects of cigarette smoking on the population at large have been well documented (54), there is a paucity of information concerning possible mechanisms of cigarette smoke damage on the lung. This is especially true regarding interacting effects of cigarette smoke and lung antioxidant defense systems such as lung NADPH, glutathione and glutathione peroxidase systems and on the important potential interacting effects with lung lysosomal enzyme systems including those of the pulmonary alveolar macrophages. There is virtually no data concerning in vivo biological assessments of the contribution of vapor plus particulate phase components of cigarette smoke on aspects of lung metabolism vs effects of vapor phase only. Likewise, although there have been a number of studies suggesting important interactions between cigarette smoking and air pollution exposure, there is little firm data available that would scientifically establish whether or not these two agents would behave synergistically insofar as their effects on lung parenchymal biochemical response are concerned or insofar as lung antimicrobial responses are concerned.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The investigators have at their disposal four laboratory areas: i) the 500 square foot Pulmonary Disease Research Laboratory at the U.C. Davis School of Medicine. This laboratory is equipped to perform cigarette exposures and biochemical assays. ii) The 400 square foot Infectious Disease Research Laboratory at the U.C.D. School of Medicine. This laboratory is equipped to assess pulmonary antibacterial defense function. iii) Three adjacent 250 square foot laboratories at the U.C. Primate Research Center. These laboratories are equipped to perform tissue preparation, ultracentrifugation, and biochemical assays. iv) The 4,000 square foot Air Pollution Exposure Facility at the U.C. Davis Primate Research Center. This facility is equipped for all aspects of exposure of laboratory animals to air pollution.

11. Additional facilities required:

Should the proposed studies be approved by the Council it would be most desirable if we could obtain an additional Cigarette Smoke Exposure Apparatus for our animal exposures. This would be our highest priority. While the Walton Chamber is satisfactory, it is far from ideal. We would like to obtain, as a minimum, one of the modified revised Walton Chambers and most ideally we would like to obtain a chamber that would allow for more simultaneous exposures than can be presently accomplished (6 rats at a time).

There are no other major space or equipment needs to accomplish the proposed studies. The budget equipment items mentioned are all relatively minor. A table top centrifuge is requested to facilitate tissue processing. The present Walton apparatus requires certain modifications that are estimated to cost \$2,000. Lastly, certain nebulizer modifications are required for assessment of the rat antibacterial defense capabilities.

12. Biographical sketches of investigator(s) and other professional personnel (append).

see attached c.v.s

13. Publications: (five most recent and pertinent of investigator(s), append list, and provide reprints if available).

see attached

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

* Carroll E. Cross, M.D. 10
 * Elliot Goldstein, M.D. 5
 Mohammed G. Mustafa, Ph.D. 40
 Ching K. Chow, Ph.D. 80
 George K. York, Ph.D. candidate in Path. 100
 Ph.D. candidate in Microbiology 25
 ** 4 work-study students to work as
 cigarette exposure machine tech. 20

Technical

None

* In keeping with University of California academic staff policy, 50% of research time is listed as salary

** Work-study program students are supported by \$3 in Federal grants for each \$1 in research funds.

Sub-Total for A

REDACTED

B. Consumable supplies (by major categories)

Biochemicals \$ 1,500
 Radiophosphorus and radioiodine 1,000
 Histochemical supplies 500
 Microbiological supplies 1,000
 Standard reference cigarettes @\$10/1000 200

Sub-Total for B

\$ 4,200

C. Other expenses (itemize)

1,000 rats @\$5. \$ 5,000
 Medical illustration and xerox 500
 Travel (2 meetings/year) 500

Sub-Total for C

\$ 6,000

Running Total of A + B + C

\$35,350

D. Permanent equipment (itemize)

Table top centrifuge 1,000
 Cigarette Chamber Modifications & additions 2,000
 Nebulizer equipment (hand made, specially constructed
 for bacterial aerosol exposures) 1,000

Sub-Total for D

\$ 4,000

E

5,300

E. Indirect costs (15% of A+B+C)

Total request

\$44,650

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	4,000	6,000	2,000	5,400	43,400
Year 3		3,000	3,000	1,000	3,000	24,000

5.

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Pulmonary Effects of Environmental Oxidant Pollutants	U.S. Public Health Service (ES AP 00628-01)	\$10,000*	June 1, 1971 - May 31, 1975

* This is a Program Project Grant for \$310,866. Pollution chambers and some animal costs are covered by the Core support. My personal share is approximately \$10,000/yr.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Proposed Studies of the Effects of Oxidant Pollutants on Red Blood Cells and Lung Cells	California Air Resources Board	\$40,300	October 1, 1973 - September 30, 1975

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made"

Principal investigator

Typed Name Carroll E. Cross, M.D.Signature Carroll E. Cross Date _____
 Telephon R
 Area Code Number Extension

Check payable to _____

Mailing address for checks

Responsible officer of institution

Typed Name _____

Title _____

Signature _____ Date _____

 Telephone _____
 Area Code Number Extension

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July 1, 1973

CURRICULUM VITAE

NAME Carroll Edward Cross, M.D.ADDRESS Pulmonary Disease Research Laboratory
Department of Internal Medicine
School of Medicine
University of California
Davis, California 95616DATE AND PLACE
OF BIRTH

REDACTED

MARITAL STATUS

REDACTED

EDUCATIONPomona College
University of Washington, School of Medicine,
Columbia University, College of Physicians and Surgeons -

REDACTED

MILITARY

U.S. Army National Guard,

REDACTED

BOARD STATUSDiplomate: American Board of Internal Medicine (1969)
Passed: Subspecialty Boards in Chest Medicine (1971)POSITIONS (MEDICAL)

1961 - 1962	Intern, Mixed Medical, University of Wisconsin Hospital Center, Madison, Wisconsin
1962 - 1964	Residency, Internal Medicine, Stanford University, School of Medicine, Palo Alto, California
1964 - 1967	Fellowship in Pulmonary Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
1967 - 1968	Instructor of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
1968 - 1973	Assistant Professor of Medicine, University of California at Davis, School of Medicine, Davis, California
1969 - 1973	Assistant Professor of Human Physiology, University of California at Davis, School of Medicine, Davis, California
1973 - present	Associate Professor of Human Physiology and Medicine, University of California at Davis, School of Medicine, Davis, California

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24. Mustafa, M.G., Peterson, P.A., Munn, R.J. and Cross, C.E.: Effects of Cd^{++} on metabolism of lung cells. Proceedings of the Second International Clean Air Congress, Edited by H.M. Englund and W.T. Berry, Academic Press, N.Y., p. 143-151, 1971.
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30. Robin, E.D., Cross, C.E. and Zelis, R.: Pulmonary edema: Part I, Anatomy, Physiology and Pathophysiology. New Eng. J. Med. 288: 239-246, 1973.
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PUBLICATIONS SUBMITTED

Giri, S.N., Hollinger, M.A., Dungworth, D.L. and Cross, C.E.: Effects of thiourea on pulmonary edema, pleural effusions and peritoneal effusions and toxicity in rats pretreated with actinomycin D.

DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. II. Oxidation of reduced glutathione and mixed disulfide formation between protein and non-protein sulfhydryls.

DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. III. Oxidation of reduced glutathione and mixed disulfide formation between protein and non-protein sulfhydryls.

DeLucia, A.J., Mustafa, M.G., Cross, C.E. and Asghar, K.: Increased level of non-protein sulfhydryls in ozone-exposed rodent lungs.

Mustafa, M.G. and Cross, C.E.: Studies of mammalian lung mitochondria: Effects of short-term ozone exposure on oxidative and energy metabolism.

York, G.K., Cross, C.E., Mustafa, M.G., and Bodai, B.I.: Lung glucose-6-phosphate dehydrogenase stimulation by cigarette smoke. Effects of short-term ozone exposure.

Mustafa, M.G., and Cross, C.E.: Effects of short-term ozone exposure on lung mitochondrial oxidative and energy metabolism.

Mustafa, M.G., and Cross, C.E.: Lung cell mitochondria: Rapid oxidation of glycerol-1-phosphate but slow oxidation of 3-hydroxybutyrate.

1003539506

CURRICULUM VITAE

Elliot Goldstein, M. D.

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Born: REDACTED

Education:

Cornell University - Chemistry, Zoology -

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Cornell Medical College -

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Employment:

University of California Hospital - Pathology Internship - July 1960 - June 1961.

Mount Sinai Hospital, New York - Rotating Internship - July 1961 - June 1962.

Bellevue Hospital, First (Columbia) Division - Medical Resident -
July 1962 - June 1964.

Boston City Hospital, Channing and Thorndike Fellow in Infectious Disease -
Harvard Medical Service - July 1964 - June 1966.

United States Army, Major - Medical Corp, Vietnam - July 1966-67,
Fort Devens, July 1967 - 68.

Harvard Medical School - Instructor in Medicine, Director of Tuberculosis
Laboratory, Mattapan Sanatorium, Boston City Hospital - July 1968 -
July 1969.

University of California at Davis School of Medicine - Assistant Professor of
Medicine - August 1969 - June 1971.

University of California at Davis School of Medicine - Associate Professor of
Medicine - July 1971 - present.

Society Memberships:

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Professional Activities:

- 1) Reviewer, Journal of Infectious Diseases
- 2) Consultant to the Wright-Patterson Research Laboratories, Dayton, Ohio

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PUBLICATIONS

--exclusive of abstracts--

Elliot Goldstein, M. D.

1. Goldstein, E., and Rambo, O. N.: Cryptococcal infection following steroid therapy. *Ann. Int. Med.* 56:114-120, 1962.
2. Cooper, R. A., and Goldstein, E.: Histoplasmosis of the central nervous system. *Am. J. Med.* 35:45-57, 1963.
3. Goldstein, E., and Janoski, A. H.: *Haemophilus influenzae* pyarthroses in an adult. *Arch. Int. Med.* 114:647-650, 1964.
4. Goldstein, E., Grieco, M. H., Finkel, G., and Louria, D. B.: Studies on the pathogenesis of experimental *Candida parapsilosis* and *Candida guilliermondii* infections in mice. *J. Inf. Dis.* 115:293-302, 1965.
5. Kass, E. H., Green, G. M., and Goldstein, E.: Mechanisms of antibacterial action in the respiration system. *Bact. Rev.* 30:488-496, 1966.
6. Goldstein, E.: Four important mycotic infections: Histoplasmosis, coccidioidomycosis, cryptococcosis and candidiasis - their present clinical status and significance. *Revue de information de corps medicale.* 4:377-383, 1966.
7. Goldstein, E., and Green, G. M.: Inhibition of pulmonary bacterial clearance during acute renal failure. *Antimicrobial Agents and Chemotherapy--1965. American Society for Microbiology. Ann Arbor, Michigan.* 1966. pp. 22-25.
8. Green, G. M., and Goldstein, E.: A method for quantitating intrapulmonary bacterial inactivation in individual animals. *J. Lab. Clin. Med.* 68:669-677, 1966.
9. Goldstein, E., and Green, G. M.: The effect of acute renal failure on the bacterial clearance mechanisms of the lung. *J. Lab. Clin. Med.* 68:531-542, 1966.
10. Goldstein, E., Daly, A. K., and Seamans, C.: *Haemophilus influenzae* as a cause of adult pneumonia. *Ann. Int. Med.* 66:35-40, 1967.
11. Goldstein, E., and Green, G. M.: Alteration of the pathogenicity of *Pasteurella pneumotropica* for the murine lung caused by changes in pulmonary antibacterial activity. *J. Bact.* 93:1651-1656, 1967.
12. Goldstein, E.: A clinical study of falciparum and vivax malaria in Vietnam servicemen. *Military Med.* 133:991-996, 1968.
13. Goldstein, E., and Porter, D.: Fatal thrombocytopenia with cerebral hemorrhage in mononucleosis. *Arch. Neurol.* 20:533-535, 1969.

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14. Goldstein, E., Green, G. M., and Seamans, C.: The effect of silicosis on the antibacterial defense mechanisms of the murine lung. *J. Inf. Dis.* 120:210-216, 1969.
15. Fletcher, J., and Goldstein, E.: The effect of parenteral iron preparations on renal infection due to *Escherichia coli* in rats and mice. *Antimicrobial Agents and Chemotherapy--1969*. American Society for Microbiology. Ann Arbor, Michigan. 1970. pp. 454-457.
16. Goldstein, E., Green, G. M., and Seamans, C.: The effect of acidosis on pulmonary bactericidal function. *J. Lab. Clin. Med.* 75:912-923, 1970.
17. Goldstein, E.: Review of the literature pertaining to infection within a spacecraft. In: *Infectious Disease in Manned Spaceflight: Probabilities and countermeasures*. National Academy of Science. 1970. Appendix A. pp. 102-136.
18. Goldstein, E., and Malloy, M.: Evaluation of the fluorochrome stain in detection of mycobacteria under routine laboratory conditions. *J. Conference Pub. Health Laboratory.* 28:135-145, 1970.
19. Goldstein, E., Munson, E. S., Eagle, C., Martucci, R. and Hoeprich, P. D.: Influence of anesthetic agents on murine pulmonary bactericidal activity. *Antimicrobial Agents and Chemotherapy--1970*. American Society for Microbiology. Ann Arbor, Michigan. 1971. pp. 231-235.
20. Fletcher, J. and Goldstein, E.: The effect of parenteral iron preparations on experimental pyelonephritis. *Br. J. Exp. Path.* 51:280-285, 1970.
21. Goldstein, E., Tyler, W. S., Hoeprich, P. D., and Eagle, M. C.: Adverse influence of ozone on pulmonary bactericidal activity of the murine lung. *Nature.* 229:262-263, 1971.
22. Goldstein, E., Tyler, W. S., Hoeprich, P. D., and Eagle, M. C.: The effect of ozone on the anti-bacterial defense mechanisms of the murine lung. *Arch. Int. Med.* 127:1099-1102, 1971.
23. Goldstein, E., Munson, E. S., Eagle, C., Martucci, R. W. and Hoeprich, P. D.: The effects of anesthetic agents on murine pulmonary bactericidal activity. *Anesthesiology.* 34:344-352, 1971.
24. Hahn, F. F., Goldstein, E., and Dungworth, D. L.: Effect of whole body x-irradiation on pulmonary bactericidal function. *Rad. Research.* 47:461-471, 1971.
25. Goldstein, E.: Evaluation of the role of nitrogen dioxide in the development of respiratory diseases in man. *California Medicine.* 115:21-28, 1971.

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26. Goldstein, E., Eagle, C. and La Casse, M. L.: In vitro chemotherapeutic combinations against isoniazid-resistant *Mycobacterium tuberculosis* and *Mycobacterium fortuitum*. Appl. Micro. 22:329-333, 1971.
27. Goldstein, E., and Hoeprich, P. D.: Problems in the diagnosis and treatment of systemic candidiasis. J. Inf. Dis. 125:190-193, 1972.
28. Goldstein, E., Eagle, M. C., and Hoeprich, P. D.: Influence of ozone on pulmonary defense mechanisms of silicotic mice. Arch. Environ. Hlth. 24:444-448, 1972.
29. Goldstein, E., Buhles, W. C., Akers, T. G., and Vedros, N.: Murine resistance to inhaled *Neisseria meningitidis* after infection with an encephalomyocarditis virus. Inf. & Imm. 6:398-402, 1972.
30. Goldstein, E., Winship, M. J., and Pappagianis, D.: Ventricular fluid and the diagnosis of Coccidioidal meningitis. Ann. Int. Med. 77: 243-246, 1972.
31. Goldstein, E., Lewis, Jerry P., Eagle, M. Carroll, and Hoeprich, Paul D.: Evidence for a non-marrow precursor pool of alveolar macrophages. Exper. Hemat. 22:81-83, 1972.
32. Hoeprich, Paul D., and Goldstein, Elliot: Pneumonias. Hospital Physician. (in press) 1973.
33. Eisele, John H., Goldstein, Elliot, Martucci, Richard, W., and Eagle, Carroll.: The influence of acute respiratory acidosis on the pulmonary defense mechanisms in rats. Amer. Rev. Resp. Dis. (in press) 1973.
34. Goldstein, Elliot, Eagle, M. Carroll, and Hoeprich, Paul D.: The effect of nitrogen dioxide on the antibacterial defense mechanisms of the murine lung. Arch. Environ. Hlth. 26:202-204, 1973.
35. Goldstein, Elliot, and Lewis, Jerry P.: Patterns of pulmonary alveolar macrophage function following radiation injury. J. Lab. Clin. Med. (in press) 1973.

Chapters in Books

1. Goldstein, E.: Otitis Externa, Chapter 149, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1267-1271.
2. Goldstein E.: Rat Bite Fever, Chapter 129, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1149-1152.
3. Goldstein, E., and Hoeprich, P. D.: Diphtheria, Chapter 24, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 269-278.
4. Goldstein, E.: Bartonellosis, Chapter 131, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1159-1161.
5. Goldstein, E.: Acid-fast staining, in Chapter 8, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972. P. 89.

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EDUCATION

Government Muslim High School, Dacca, Bangladesh
Matriculation R

Jagannath College, Dacca, Bangladesh, I.Sc., R

University of Dacca, Dacca, Bangladesh, I.Sc.
M.Sc. in Biochemistry, 1962 R

University of California, Berkeley, California,
M.A., in Comparative Biochemistry, R

Oregon State University, Corvallis, Oregon, Advanced
to Doctoral Candidacy in Biochemistry, R

State University of New York at Albany, Albany, New
York, Ph.D. in Biochemistry, R

POSITIONS

1962 - 1963

Teaching and Research Fellow, Department of Biochemistry
and Nutrition, University of Dacca, Dacca, Bangladesh

1963 - 1966

Research Assistant, Department of Physiology, University
of California, Berkeley, California

1966 - 1968

Research Assistant, Science Research Institute and Depart-
ment of Biochemistry and Biophysics, Oregon State
University, Corvallis, Oregon

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1968 - 1969

Research Assistant, Department of Chemistry, State University of New York, Albany, New York.

1969 - present

Assistant Research Biochemist, Departments of Biological Chemistry and Internal Medicine, School of Medicine, and California Primate Research Center, University of California, Davis, California

SCHOLARSHIPS

1961 - 1962

Departmental Scholarship, University of Dacca, Bangladesh

1963 - 1969

Fulbright Travel Grant, U.S. Educational Foundation in Pakistan (Bangladesh)

GRANTS RECEIVED

Tuberculosis and Respiratory Disease Association of California, "Pulmonary Macrophage: Effects of Common Air Pollutants and Other Noxious Inhalants on Respiration and Energy Production and Utilization Mechanisms". January 1, 1970 to June 30, 1972; \$12,600.

Co-investigator in charge of biochemical aspects of lung studies on four other funded multidisciplinary collaborative grants, viz.:

1) National Institute of Environmental Health Sciences, N.I.H., "Pulmonary Effects of Environmental Oxidant Pollutants, June 1, 1971 to June 1, 1975, \$780,000 (D. L. Dungworth).

2) American Medical Association Education and Research Foundation Committee for Research on Tobacco and Health "Tobacco Smoke and Lung Biochemistry". February 1, 1971 to February 1, 1973, \$60,000 (C. E. Cross).

3) The Council for Tobacco Research - U.S.A., "Effects of Cigarette Smoke on the Pulmonary Lymphoreticular System", January 1, 1970 to January 1, 1973, \$120,000 (C. E. Cross).

4) National Institutes of Health, "Health Science Advancement Award in Comparative Medicine", June 1, 1968 to June 1, 1973, Approximately \$5,000,000 (R. E. Stowell).

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PUBLICATIONS

1. Mustafa, M.G., Chakraborty, S., Rahman, M.M. and Ahmad, K.: Semimicro determination of uric acid in blood. Pakistan J. Biol. Agri. Sci., 6: 61-69, 1963.
2. Packer, L. and Mustafa, M.G.: Pathways of electron flow established by tetramethylphenylenediamine in mitochondria and ascites tumor cells. Biochem. Biophys. Acta, 113: 1-12, 1966.
3. Mustafa, M.G., Utsumi, K., and Packer, L.: Damped oscillatory control of mitochondrial respiration and volume. Biochem. Biophys. Res. Commun., 24: 381-385, 1966.
4. Packer, L., Utsumi, K., and Mustafa, M.G.: Oscillatory states of mitochondria: I. Electron and energy transfer pathways. Arch. Biochem. Biophys. 177: 381-393, 1966.
5. Mustafa, M.G. and King, T.E.: Wurster's blue mediated oxidation of NADH and phosphorylation in mitochondria. Arch. Biochem. Biophys., 122: 501-508, 1967.
6. Mustafa, M.G., Cowger, M.L. and King, T.E.: On the energy-dependent bilirubin-induced mitochondrial swelling. Biochem. Biophys. Res. Commun., 29: 661-666, 1967.
7. Mustafa, M.G., Cowger, M.L., Labbe, R.F. and King, T.E.: General nature of "Wurster's blue shunts" in the respiratory chain. J. Biol. Chem., 243: 1908-1918, 1968.
8. Mustafa, M.G., Cowger, M.L. and King, T.E.: Effects of bilirubin on mitochondrial reactions. J. Biol. Chem., 244: 6403-6414, 1969.
9. Mustafa, M.G., Ibrahim, A.B., Le, C.T. and Cross, C.E.: Pulmonary alveolar macrophage: Demonstration of $\text{Na}^+\text{-K}^+$, Mg^{++} ATPase activity. Life Sci., 8: 1343-1351, 1969.
10. Mustafa, M.G. and King, T.E.: Binding of bilirubin with lipid: a possible mechanism of its toxic reaction in mitochondria. J. Biol. Chem., 245: 1084-89, 1970.
11. Mustafa, M.G., and Cross, C.E.: Localization of $\text{Na}^+\text{-K}^+$, Mg^{++} ATPase in alveolar macrophage subcellular fractions. Life Sci., 9: 947-54, 1970.
12. Cross, C.E., Ibrahim, A.B., Ahmed, M., and Mustafa, M.G.: Effects of Cd^{++} on respiration and ATPase activity of the pulmonary alveolar macrophage: A model for the study of environmental interference with pulmonary cell function. Environ. Res., 3: 512-520, 1970.
13. Meyer, D.H., Cross, C.E., Ibrahim, A.B. and Mustafa, M.G.: Nicotine effects on alveolar macrophage respiration and adenosine triphosphatase activity. Arch. Environ. Health, 22: 362-365, 1971.

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14. Mustafa, M.G., Cross, C.E., and Tyler, W.S.: Interference of cadmium ion with oxidative metabolism of alveolar macrophages. Arch. Int. Med., 127: 1050-1058, 1971.
15. Cross, C.E., Mustafa, M.G., Peterson, P., and Hardie, J.A.: Pulmonary alveolar macrophage: Membrane associated Na^+ , K^+ , Mg^{++} -adenosine triphosphatase system. Arch. Int. Med., 127: 1069-1077, 1971.
16. Mustafa, M.G., Peterson, P.A., Munn, R.J., and Cross, C.E.: Effects of Cd^{++} on metabolism of lung cells. In Proceedings of the Second International Clean Air Congress, Edited by H.M. Englund and W.T. Berry, Academic Press, N.Y., p. 143-151, 1971.
17. Mustafa, M.G., and Cross, C.E.: The pulmonary alveolar macrophage: Oxidative metabolism of isolated cells and mitochondria and effect of cadmium ion on electron and energy transfer reactions. Biochemistry, 19: 4176-4185, 1971.
18. Mustafa, M.G., Cross, C.E., Munn, R.J., and Hardie, J.A.: Effects of divalent metal ions on alveolar macrophage membrane adenosine triphosphatase activity. J. Lab. Clin. Med., 77: 563-571, 1971.
19. Utsumi, K., Torres-Pereira, Mustafa, M.G., and Oda, T.: Change of proton gradient in mitochondria at various energy states. Acta Med. Okayama, 25: 493-504, 1971.
20. Mustafa, M.G., Le, C.T., Harris, N.E., and Cross, C.E.: Effects of proteases and lipases on membrane associated biochemical reactions of alveolar macrophage and lung tissue. In Pulmonary Emphysema and Proteolysis, C. Mittman, Editor, Academic Press, N.Y., p. 281-294, 1972.
21. DeLucia, A.J., Hoque, P.M., Mustafa, M.G., and Cross, C.E.: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. J. Lab. Clin. Med., 80: 559-566, 1972.
22. York, G.K., Arth, C., Stumbo, J.A., Cross, C.E. and Mustafa, M.G.: Effect of cigarette smoke and tobacco extract on lung macrophage respiration. Arch. Environ. Health, in press, 1973.
23. Mustafa, M.G., DeLucia, A.J., York, G.K., Arth, C. and Cross, C.E.: Ozone interaction with rodent lung. II. Effects on oxygen consumption of mitochondria. J. Lab. Clin. Med., in press, 1973.
24. Mustafa, M.G., DeLucia, A.J. and Cross, C.E.: Effect of ozone exposure on lung mitochondrial oxidative metabolism. Chest, in press, 1973.

1003539514

Manuscripts submitted or in preparation (1973)

1. DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. III. Oxidation of reduced glutathione and mixed disulfide formation between protein and nonprotein sulfhydryls.
2. DeLucia, A.J., Mustafa, M.G., Cross, C.E. and Asghar, K.: Increased level of nonprotein sulfhydryls in ozone-exposed rodent lungs.
3. Mustafa, M.G. and Cross, C.E.: Studies of mammalian lung mitochondria: Effects of short-term ozone exposure on oxidative and energy metabolism.
4. Mustafa, M.G., DeLucia, A.J., Cross, C.E., Dungworth, D.L. and Tyler, W.S.: Biochemical and morphological alterations in the lung following ozone exposure.
5. Cytoplasmic and microsomal membrane fractions of lung: Adenosine triphosphatase activity.
6. Lung monoamine oxidase. I. Substrate utilization and subcellular localization. II. Effect of acute and chronic ozone exposure.
7. Lung mitochondria: Increased oxidation of glycerol-1-phosphate in chronic ozone exposure.
8. Increased mitochondrial enzymatic activities in the lung during recovery from acute ozone exposure.
9. Increased enzymatic activities for sulfhydryl metabolism activities in the lung in chronic ozone exposure.
10. Biological effects of atmospheric pollutants, a chapter for the book on "Atmospheric Sciences".

1003539515

ABSTRACTS

1. Mustafa, M.G. and Packer, L.: Interaction of tetramethylphenylenediamine with rat liver mitochondria. Pacific Slope Biochemical Conference (Presented at August meetings at Eugene, Oregon, 1966).
2. Mustafa, M.G. and King, T.E.: Wurster's blue mediated oxidation of NADH and associated phosphorylation in mitochondria. Pacific Slope Biochemical Conference (Presented at June meetings at Davis, California, 1967).
3. Mustafa, M.G.: General nature of the interactions of TMPDox with the mitochondrial respiratory chain. Pacific Slope Biochemical Conference (Presented at June meetings at Davis, California, 1967).
4. Mustafa, M.G. Cowger, M.L. and King, T.E.: Energy-dependent bilirubin-induced swelling. Fed. Proc., 27, 833, 1968.
5. Mustafa, M.G.: Mechanism of bilirubin action affecting processes manifested by mitochondrial membrane. Fed. Proc., 28, 822, 1969.
6. Mustafa, M.G., Ibrahim, A.B., Tyler, W.S. and Cross, C.E.: Pulmonary alveolar macrophage: Demonstration of $\text{Na}^+\text{-K}^+$, Mg^{++} ATPase activity. Fed. Proc., 29, 663, 1970. (Presented at FASEB American Physiologic Society, April 12-17 meetings at Atlantic City).
7. Mustafa, M.G., Ibrahim, A.B., Hance, A.J. and Cross, C.E.: Pulmonary alveolar macrophage: Inhibition of electron and energy transfer by cadmium ion in whole cells and mitochondria. Fed. Proc., 29, 911, 1970.
8. Cross, C.E., Ibrahim, A.B., and Mustafa, M.G.: Pulmonary alveolar macrophage: Effects of divalent cations on respiratory and energy transfer systems. Clin. Res., 18, 483, 1970.
9. Mustafa, M.G., T.S. Groves and Cross, C.E.: Contraction and aggregation phenomena in pulmonary alveolar macrophages (PAMs). Clin. Res., 19, 138, 1971.
10. Cross, C.E., Le, C.T. and Mustafa, M.G.: Effects of ozone on some pulmonary alveolar macrophage (PAM) biochemical reactions. Clin. Res., 19, 147, 1971.
11. Mustafa, M.G. and Cross, C.E.: Oxidative metabolism of pulmonary alveolar macrophages. Eighth National Scientific Meeting of the Reticuloendothelial Society, 1971. (Presented at November 30-December 3 meetings at Detroit).
12. Mustafa, M.G., Cronin, S.R., Duvall, T.R. and Cross, C.E.: Ozone effect on activities of lung cellular membrane enzymes. Clin. Res. 20: 197, 1972.
13. DeLucia, A.J., Hoque, P.M., Cross, C.E. and Mustafa, M.G.: Sulfhydryl content of rodent lungs under oxidant stress. Clin. Res. 20: 240, 1972.

1003539516

14. Mustafa, M.G., Belleci, P. and Cross, C.E.: Oxidation of biological amines by mammalian lung tissue. Fifth International Congress of Pharmacology, 1972 (Presented at July 23-28 meetings at San Francisco).
15. Mustafa, M.G. and Cross, C.E.: Effect of tobacco smoke on lung cellular membrane enzymes. Committee for Research on Tobacco and Health, American Medical Association Education and Research Foundation, Third Research Conference, 1972. (Presented at May 7-9 meetings at Newport Beach, Calif.).
16. DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Glutathione and sulfhydryl-containing enzyme activities in lung under ozone stress. Clin. Res. 21, 223, 1973.
17. Mustafa, M.G., Cross, C.E. and Tyler, W.S.: Ozone exposure causing biochemical and anatomical alterations in the lung. Fed. Proc. 32, 864, 1973. (Presented at FASEB American Pathological Society, April 15-20 meetings at Atlantic City).

1003539517

DISSERTATIONS

1. Mustafa, M.G.: On an antiuricemic principle from *Boerhaavia diffusa* linn -- a dissertation for Master of Science in Biochemistry submitted to the University of Dacca, Dacca, Bangladesh (1962).
2. Mustafa, M.G.: Oxidation of tetramethylphenylenediamine by mitochondria -- a dissertation for Master of Arts in Comparative Biochemistry submitted to the University of California, Berkeley, California (1966).
3. Mustafa, M.G.: Certain electron and energy transfer reactions of mitochondria -- a dissertation for Doctor of Philosophy in Biochemistry submitted to the State University of New York at Albany, Albany, New York (1969).

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EDUCATION

National Taiwan University, Taipei, Taiwan. REDACTED in Ag. Chemistry.
 Univ. of Illinois, Urbana, Illinois, REDACTED in Animal Nutrition.
 Univ. of Illinois, Urbana, Illinois, REDACTED in Nutritional
 Sciences, minors in Biochemistry and Food Science.

RESEARCH AND PROFESSIONAL EXPERIENCE

1972-present, Assist. Research Biochemist, Univ. of Calif., Davis, Calif.
 Oxidant induced lipid peroxidation damage; antioxidant function of
 SH- compounds, Se and vitamin E; relationship between Se and glutathione
 peroxidase; mechanism of lipid peroxidation associated enzyme alteration;
 automation of enzyme assay; enzyme purification; lipid peroxidation re-
 lated aging (fluorescent) pigments and pathogenic changes.

1971-1972, National Vitamin Foundation Fellow, Univ. of Calif., Davis, Calif.
 Air pollution oxidant damage and antioxidant protection; enzymatic
 protection against lipid peroxidation damage; tissue enzyme distribution;
 enzymes of carbohydrate metabolism; alteration of enzymic activity as
 a sensitive indicator for tissue damage; lipid peroxide preparation and
 detoxification mechanism.

1970-1971, Research Associate, Univ. of Illinois, Urbana, Illinois
 Turn-over rates of tocopherols; toxicity of NO_2 and nitrate (and
 nitrite); Mississippi Holmes County Nutrition Survey; phospholipids,
 sterols and carotenoid compounds on the stability of oils.

1969-1970, Assoc. Research Scientist, New York Univ. Medical Center, N. Y.
 Assay, absorption, transportation and metabolism of α -tocopherol in
 human blood; lipid composition of erythrocyte membranes.

1964-1969, Graduate Research Assistant, Univ. of Illinois, Urbana, Illinois
 Chemistry and biochemistry of tocopherols and related compounds; syn-
 thesis of radioactive tocopherols and tocopheryl quinone; methods for
 lipid and tocopherol assay; isolation and identification of organic
 compounds; oxidative stability of tocopherols fatty acids and oils;
 preparation of hydroperoxide and hydroxy fatty acids, and metabolism;
 vitamin A deficiency and oxidative phosphorylation; lipid biosynthesis
 in root tissue.

1963-1964, Researcher, Subsistence Factory, CSF, Taichung, Taiwan
 Inspection, research and development of canned foods and combat rations.

1962-1963, Senior thesis research, National Taiwan Univ., Taipei, Taiwan
 Protein and amino acid nutrition.

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HONORS

Research assistantship, Univ. of Illinois, 1964-1969; American Society for
 Clinical Nutrition Research Fellowship, 1971-1972; Sigma Xi Grant-in-aid
 for Research Award, 1972; listed in American Men & Women of Science.
 Members of Sigma Xi and New York Academy of Sciences.

List of Publications

1. Chow, C. K. Effect of d,l-methionine supplementation on growth, nitrogen retention and energy metabolism of the growing rats. B.S. thesis, National Taiwan University, 1963.
2. Chow, C. K. Metabolism of α -tocopheryl quinone and α -tocopheryl hydroquinone. M.S. thesis, University of Illinois, 1966.
3. Chow, C. K. Studies on the assay, stability, antioxidant activity and oxidation products of tocopherols. Ph.D. thesis, University of Illinois, 1969. Diss. Abstr. Int. B. 31, 559, 1970.
4. Chow, C. K., H. H. Draper, A. S. Csallany and M. Chiu. The metabolism of C-14- α -tocopheryl quinone and C-14- α -tocopheryl hydroquinone. *Lipids* 2, 390, 1967.
5. Chow, C. K., H. H. Draper and A. S. Csallany. Method for the assay of free and esterified tocopherols. *Anal. Biochem.* 32, 81, 1969.
6. Chow, C. K. and H. H. Draper. Effect of artificial drying on the tocopherols and fatty acids of corn. *J. Agri. Food Chem.* 17, 1316, 1969.
7. Chow, C. K. and H. H. Draper. Isolation of γ -tocotrienol dimers from *Hevea latex*. *Biochemistry* 9, 445, 1970.
8. Chow, C. K. and H. J. Kayden. Tocopherol levels in human erythrocytes. *Fed. Proc.* 29, 693, 1970abs.
9. Chow, C. K., A. S. Csallany and H. H. Draper. Turnover rates of tocopherols in rabbit plasma. *Nutr. Report Int'l.* 4, 45, 1971.
10. Csallany, A. S., C. K. Chow and H. H. Draper. Relationship between erythrocyte and plasma vitamin E in the rabbit. *Nutr. Report Int'l.* 4, 325, 1971.
11. Fletcher, B. L., K. Reddy, C. J. Dillard, C. K. Chow and A. L. Tappel. Fluorescence measurement of lipid peroxidation and the effect of dietary vitamin E on biochemical damage in rats exposed to O_3 and NO_2 . *Fed. Proc.* 31, 713, 1972abs.
12. Chow, C. K. and A. L. Tappel. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids* 7, 513, 1972.
13. Chow, C. K. and A. L. Tappel. Glutathione peroxidase system protects against oxidative damage in lungs of ozone-exposed rats. *Clin. Res.* 21, 277, 1973abs.
14. Chow, C. K. and A. L. Tappel. Activities of pentose shunt and glycolytic enzymes in lungs of ozone-exposed rats. *Arch. Environ. Health*, 26, 618, 1973.

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List of Publications (continued)

15. Chow, C. K., K. Reddy and A. L. Tappel. Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. *J. Nutr.* 103, 618, 1973.
16. Chow, C. K. On the nitrates, nitrites and nitrosamines in foods. *Food Industries (Taiwan)*, in press, 1973.
17. Kayden, H. J. and C. K. Chow. Spectrophotometric method for the determination of α -tocopherol in red blood cells. ~~XXXXXXXXXX~~ *J. Lipid Research*, IN Press, 1973.
18. Chow, C. K., C. J. Dillard and A. L. Tappel. Glutathione peroxidase system and lysozyme in rats exposed to ozone and nitrogen dioxide. Submitted to *Environ. Res.*
19. Smith, P, A. L. Tappel and C. K. Chow. Glutathione peroxidase activity as a function of dietary selenomethionine. Submitted to *Science*.
20. Chow, C. K. On the existence of lipid peroxides in vivo and biological function of vitamin E. Submitted to *J. Chinese Agri. Chem. Soc.*
21. Chow, C. K. and A. L. Tappel. Kinetic and mechanism of glutathione peroxidase activity in rat tissues as influenced by dietary selenomethionine. In preparation for *J. Nutr.*
22. Chow, C. K. and A. L. Tappel. Normal glutathione peroxidase activity in Batten's syndrome. In preparation for *Nature*.
23. Chow, C. K. and H. H. Draper. Oxidative stability of tocopherols in vegetable oils. In preparation for *J. Am. Oil Chemists' Soc.*

1003539521

July 24, 1973

MEMORANDUM FOR FILE

SUBJECT: Grant 734R2. (10937)
Carroll E. Cross, M.D.
"Effects of Tobacco Smoke on the Pulmonary Alveolar Macrophage -
Cytokinetic, Phagocytic Activities and Biochemistry."
Site Visit: July 9, 1973.

The co-investigators I saw were Elliot Goldstein, M.D., internist in charge of infectious diseases who trained at Channing, and was Huber's predecessor, and Mohammed G. Mustafa, Ph.D., trained in biochemistry. W. S. Tyler, formerly Professor of Anatomy in the veterinary school is now Director of the Primate Biology Laboratory and is also a collaborator indirectly. Mustafa and Cross have space there. Weekly seminars are held on pulmonary function problems for staff communication and for instruction of graduate and medical students taking research work. Actually one undergraduate premed was also working on monoamino oxidases in lysosomes. A general sense of collaboration was apparent. The medical departments were crowded in temporary laboratories. Construction of the basic medical sciences buildings will not begin until March of 1975. With 100 medical students per class the facilities will be crowded. Clinical facilities are now in Sacramento Hospital about 15 miles away. These facilities will be used until Medical Sciences II is constructed which will have 300-350 beds.

I first saw the Cross laboratories in the medical school and saw two students working there, one an undergraduate and the other, George York, a graduate. The latter was undertaking the experiment outlined for continuation.

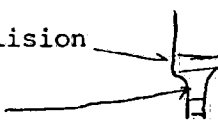
The Waltham was operating on 1 cigarette with 6 rats in holders that were much too large (guinea pig size for 35-45 gram rats, that were either with tails out of the groove and 2 were with noses in the tail groove. This was somewhat embarrassing. I don't think there is much of a constant dose here. The animal holders must be improved. The noses of the rats project too far into the chamber to permit the fan to work. Cross was anxious to have his men see a well-designed holder in function. He really needs holders of 2 sizes because of the ages at which he starts his animals. He does this because his other experiments on environmental health uses animals 1 week past weaning. Rats are pathogen-free at start.

Later I saw Ernie Bodai who was also smoking rats. He was using setup with Cambridge filters. Philip Morris cigarettes. Again I mentioned the reference cigarettes. Cross should be written about availability and costs of reference cigarettes. Bodai has his own filter -- a clever design of rapid propulsion and collision of particulates that seems to do a good job of particulate removal. It also traps H₂O and probably also H₂O-soluble gases.

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disc for acceleration and collision

filter to catch particulates



I repeated what Cross had been told that this was outside the area of cancer of the CTR. Bodai seemed an unusually bright and well-organized person.

Walter S. Tyler, Director, Davis Primate Center. He is a competent anatomist, em. and fem. person and has some excellent freeze etch specimens. His special area is studies on lung. He assumes that capillaries of bronchial arteries terminate in alveoli in some instances, differing from Liebow. The upper and lower lobes differ with more arterial perfusion of the upper lobes than the lower. Twelve years ago in Amer. J. Anat. he reported upper lobe vascularization with nicotine effects. He has now 2 freeze etch setups and 2 people to run them. He expends 40% of his time in laboratories.

Tyler is now working on Clara cells. They differ in different species. Rhesus monkey is much unlike man. The stub-tail monkey was most like man. Primate lab has 35 stub-tails on loan and is getting them back.

Dr. Neuratter, D.V.M., formerly at NIH is now at Davis as Associate Director and does all business administration.

Core facilities include virology, bacteriology, radiology, clinical chemistry, necropsy service, em. and sem. 80% of hard money goes to personnel.

Divisions:

Dr. Chapman, Behavioral studies.

Andy Hendricks, Paranatal biology.

Ernest Gardner, Congenital anomalies.

Dan Dunsworth, Pathology, including parasitology.

Oncology is being phased out. They have given their monkey lymphoma virus to other labs.

Nutrition nil now.

Genetics is interested in it from an animal breeding aspect. Blood group marker as a genetic starter.

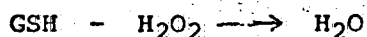
Cross and Mustafa program. A student, Nancy Brunstater (?) developed ways of washing macrophages from mouse lungs and with marker chromosomes found that (1) bone marrow provides predecessors of lung macrophages, (2) that lung macrophages are viable for 3-4 weeks, and (3) that the lung macrophages may replenish themselves.

High O₂ consumption in lung macrophages, DPN dependent. Glucose depresses O₂ consumption. He reviewed the tobacco-water (saline) and the smoke in saline experiments. The latter were particularly toxic and decreased O₂, probably gas phase substances.

Similar O₂ depression is common to many toxic substances. Suggested that this might be a way to test for toxic cigarette exposure. (1) O₂, (2) O₃ & NO₂, (3) drugs - chemotherapeutic, (4) herbicides - paraquat, (5) cigarette smoke, and (6) circulating toxins.

Heinze body anemias, red cell effects of oxidants are also known. Modification of pentose shunt cause Heinze bodies.

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GSH - GSSG - differs from cell.

The working hypothesis is that anti-oxidants might prevent non-specific damage of oxidants.

Tony DeLucca reviewed effects of GSH on O₃ exposure at toxic levels 2-4 ppm. Nothing protects against high doses. Small doses 0.8 ppm. increases SH in lung beginning at 2 days and persisting for 30 days.

Cross proposes to study enzymes in membraneous fraction and in cytosol to determine SH dependent protection. Mustafa stated that the cytochrome systems should be investigated.

Dr. Chow, Ph.D. Biochemist, is working on lipid metabolism particularly lipid proxides and peroxidases. A bioassay system has been developed.

Dr. Elliot Goldstein. His major interest is infectious diseases and he would include emphysema of some types here -- infectious complication of damaged lung tissue. He is working on macrophage effects on bacterial survival. Using staff he found normal macrophages inactivate bacteria quickly and stop division. Damaged macrophages do phagocytize but do not stop bacterial division. Clumps of bacteria occur in macrophages. He has an improved nebulizer -- over the Channing one -- a rather large apparatus. He has one graduate student working with him.

This is a multifaceted program with Carroll Cross as primary investigator. The people with him seem able and he is attracting some excellent students to work with him. He does need advice and direction on the use of the Waltham smoke exposure apparatus. I think it would be desirable to put on a demonstration for all investigators using them at the most recent possible date. Also animal holders should be designed for rats of different sizes. Even if the Waltham operates mechanically most effectively the animal holders will reduce effectiveness.

W.U.G.

WUG:ek

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#919 - DANIELS

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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

July 6, 1973

Grant application No. 919

TO: The committee comprising Drs. Loosli, Meier and Wyatt

SUBJECT: John R. Daniels, M.D., Stanford University, California
New application No. 919
"Interstitial Fibrosis of the Lung. Development of a Mouse
Model System."

History

This proposal was case #181. The vote by the Executive Committee was split, and additional information was requested. When Dr. Daniel was asked for this information, it developed that he then had the substance of an application drafted, and wished to apply formally, with the understanding that we offer no commitment, indeed no encouragement.

Application #919 requests \$51,216 plus two additional years.

Document Submitted

Attached is application dated June 21, 1973 (23 pages).

Comment

This application appears to consist largely of extracts from a NIH Program Project application, described on page 5.

FWN:gh

Encl.

FWN
F.W.N.

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

110 EAST 50TH STREET
NEW YORK, N. Y. 10022
(212) 421-8985

JUL 3 1973

Application for Research Grant
(Use extra pages as needed)

Date: June 21, 1973

1. Principal Investigator (give title and degrees):

John R. Daniels, M.D.
Assistant Professor of Medicine

2. Institution & address:

Stanford University
Stanford, California 94305

3. Department(s) where research will be done or collaboration provided:

Department of Medicine
Department of Radiology
Department of Pathology

4. Short title of study:

Interstitial Fibrosis of the Lung. Development of a Mouse Model System

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: Three (3) years

7. Brief description of specific research aims:

The specific goals of this study may be summarized as follows:

1. Characterization and comparison of lung collagens and the collagen of fibrosis
 - a. Isolation by chromatography
 - b. Cyanogen bromide peptide maps and amino acid analysis
2. Evaluation of therapeutic intervention in lung fibrosis
 - a. Lathrogen (BAPN)
 - b. Corticosteroid
3. Refinement of the model of lung fibrosis following radiation injury
 - a. Improvement of reproducibility by attention to bacteriologic control
 - b. Development of a reliable and sensitive biochemical measure of pathologic fibrosis
 - c. Detailed correlation of morphologic, biochemical and functional alterations

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8. Brief statement of working hypothesis:

2.

It is proposed to study a model of pulmonary radiation fibrosis in mice. Observed structural changes will be correlated with measurements of functional capacity and detailed biochemical analysis. These correlative studies should provide the background for systematically exploring therapeutic manipulations. Initial experimental questions include the following:

- 1) The time course and radiation dose relationship for the development of fibrosis will be determined.
- 2) Biochemical measures of total collagen content will be assessed and correlated with the pathologic appearance.
- 3) Pulmonary function (measures of compliance) will be obtained and an attempt made to correlate microscopic, functional and biochemical changes.

The therapeutic effects of modifications of collagen synthesis will be explored looking at survivorship, morphology, compliance and the biochemical expression of fibrosis. A biochemical, structural description of lung collagen will be undertaken and compared with the collagen involved during pathologic fibrosis.

9. Details of experimental design and procedures (append extra pages as necessary)

See appended pages

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Personal laboratory space is 400 square feet of standard design. It is generally well equipped and well stocked. Common equipment facilities and controlled temperature rooms are in adjacent space. Personal and common equipment capabilities include extensive column chromatographic equipment, spectrophotometer, recorder, pH meter, flash evaporator, CO₂ incubator, laminar flow hood, preparative and analytical ultracentrifuges, scintillation counters, amino acid analyser, high voltage electrophoresis, acrylamide gel electrophoresis, sonifier and a variety of partition chromatographic apparatus. Animals will be housed (as noted in Methods) in a new barrier facility under strict bacteriological control.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

See appended pages

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See appended pages

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14. First year budget:

A. Salaries (give names or state "to be recruited")

% time

Amount

Professional (give % time of investigator(s)
even if no salary requested)

DANIELS, John R. - Principal Investigator

35

BROWN, John Martin - Co-Investigator

10

FAJARDO, Luis F. - Co-Investigator

2

Technical

To be recruited - Research Technician

100

To be recruited - Dishwasher

20

To be recruited - Secretary

20

FRINGE BENEFITS

Sub-Total for A

B. Consumable supplies (by major categories)

Animals (\$4,500. See attached Budget Justification)

Chemicals

Glassware

Disposables

Chromatographic Supplies

Sub-Total for B

\$ 7,000.

C. Other expenses (itemize)

Maintenance Contracts

600.

Travel

1,000.

Publication Costs

300.

Histologic Preparations

1,000.

Sub-Total for C

\$ 2,900.

Running Total of A + B + C

\$34,971.

D. Permanent equipment (itemize)

Amino Acid Analyser Automation

5,000.

UV Monitor with 310 mμ capability

3,000.

Fraction Collector

1,000.

Recorder

700.

Constant Temperature Circulator

700.

Pump (2)

600.

Sub-Total for D

\$11,000.

E. Indirect costs (15% of A+B+C)

E

5,245.

Total request

\$51,216.

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	\$7,000.	\$3,100.	0	\$5,523.	\$42,344.
Year 3		\$7,500.	\$3,300.	0	\$5,884.	\$45,112.

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Mammalian Collagenase and Basement Membrane Collagen	NIH #AM 14896-03	\$26,177.	2/1/73 - 1/31/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Cancer and Bone Metabolism	American Cancer Society	\$132,903.	1/1/74 - 12/31/76
Laboratory Studies: Sup- port of Clinical Cancer Center (Program Project Grant Application)	NIH/NCI	\$700,245.	1/1/74 - 12/31/76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Kenneth D. Creighton
Deputy Vice President for Business and Finance
Mailing address for checks
Stanford University
Stanford, California 94305

Principal investigator

Typed Name John R. Daniels
Signature John R. Daniels Date 6/21/73
Telephone 415 321-1200 5878
Area Code Number Extension

Responsible officer of institution

Typed Name Kathleen C. Butler
Title Sponsored Projects Officer
Signature Kathleen C. Butler Date 6/19/73
Telephone (415) 321-2300 Ext 7883
Area Code Number Extension

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Budget Justification

Personnel

Salary increases of 5.5% per year are calculated.

In accordance with University policy, salary support for Dr. Daniels is requested at a fractional level commensurate with anticipated effort. No support is requested for Drs. Brown, Fajardo.

A research technician is budgeted to assist in irradiation of animals, preparation of tissues and all of the biochemical studies. A part-time equivalent secretary is budgeted to purchase supplies, keep books and type correspondence and manuscripts related to this proposal. A part-time equivalent dishwasher is budgeted for washing glassware.

Capital Equipment

Chromatographic equipment. Biochemical characterization of collagen (see Methods of Study) will involve extensive column chromatography of peptides requiring monitoring in the far UV (310 mμ to 330 mμ). The funds will cover purchase of spectrophotometer, flow cells, recorder, pump, fraction collector and constant temperature circulator.

Amino acid analyser automation. Upgrading of an existing analyser will accommodate the increased utilization involved in column determination of hydroxyproline. Specific changes include automated multiple sample applicator, programming for unattended operation and recording of digitalized data to allow computer processing.

Supplies

The cost of animals is a function of the large number which we anticipate using as well as the cost of maintaining barrier conditions for bacteriologic control.

1003539532

INTERSTITIAL FIBROSIS OF THE LUNG. DEVELOPMENT OF A MOUSE MODEL SYSTEM.

Introduction

The characteristic pathologic expression of several human disease states results from unfavorable proliferation of connective tissue (Rhodin, 1967). In such cases scarring of the organ rather than parenchymal cell destruction results in diminished functional capacity. There are several examples of this in human parenchymal disease, but among the most common clinically is interstitial pulmonary fibrosis (Spencer, 1968). Similar alterations in other organs include contractures of skin following chemical or thermal destruction (Peacock et al., 1968), the immobilization by fibrotic adhesions of reconstituted tendons, esophageal stricture following chemical irritation (lye ingestion or gastric reflux) (Davis, 1971), and hepatic cirrhosis with portal hypertension (Orloff et al., 1967; Rubin and Hutter, 1967). Interstitial fibrosis of the lung results from limited injury which may be caused by infection (bacterial, viral, mycoplasma, rickettsia), dusts, chemical irritation, circulatory abnormalities, radiation and perhaps immunologic mechanisms (Spencer, 1968). Fibrosis may follow from several different sites of primary injury: 1) organization of intra-alveolar exudates as seen following lobar pneumonia; 2) alveolar epithelial damage as seen following accretion of dust within macrophages, penetration of alveolar epithelium with fine dusts or as a consequence of hypersensitivity reactions; 3) alveolar capillary damage as seen in sclerosing alveolitis, rheumatoid disease, systemic sclerosis and radiation injury (see below); and 4) chronic lymphedema as seen in mitral stenosis.

Fibrosis as a late manifestation of the response to radiation injury is readily apparent in several tissues and may be the dominant mode of toxicity. Among those tissues in which these radiation induced alterations are of clinical importance are lung (Bennett et al., 1969), myocardium, pericardium (Steward et al., 1967; Fajardo, 1970) and bowel.

Pulmonary radiation fibrosis is readily produced in experimental animals (Jennings and Arden, 1961; Jennings and Arden, 1962). It thus serves both as a specific model for the commonly encountered clinical problem of radiation injury and as a general model to study pulmonary fibrosis and by extension the problem of pathologic fibrosis in all tissues. Work to date has primarily been descriptive. We propose to extend these studies by correlating observed structural changes with measurements of functional capacity and detailed biochemical analysis. These correlative studies should provide the background for systematically

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exploring potential therapeutic manipulations. Several direct experimental questions will be proposed initially: 1) the time course and radiation dose relationship for the development of fibrosis will be determined; 2) biochemical measures of total collagen content will be assessed and correlated with the pathologic appearance; 3) pulmonary function (measures of compliance) will be obtained and an attempt made to correlate microscopic, functional and biochemical changes. The therapeutic effects of modifications of collagen synthesis will be explored looking at survivorship, morphology, compliance and the biochemical expression of fibrosis. A biochemical, structural description of lung collagen will be undertaken and compared with the collagen involved during pathologic fibrosis.

Background

Pulmonary Morphology Following Radiation Injury

Clinical recognition of pulmonary injury by therapeutic radiation dates to 1898 (Bergonie and Tessier, 1898). Early reports emphasized compromised pulmonary function, increased densities on chest X-ray and fibrosis on autopsy specimens (Hines, 1922). In a review of material obtained from an NIH Cooperative Lung Study (Bennett et al., 1969), in 6 of 72 examined specimens the patients were thought to have died with radiation pneumonitis as the immediate or major contributing cause of death. No single pathognomonic morphologic feature was identified by examination by light microscopy. The diagnosis was made from a combination of alveolar septal thickening, proliferation and desquamation of atypical septal cells, hyaline membrane formation and pulmonary vascular changes. Alveolar cell hyperplasia with bizarre cytopathy was perceived as the most specific change for radiation injury. Alveolar septal thickening initially consisted of edema and mononuclear infiltrate which then evolved to fibrillar thickening and eventually to dense collagenization. Small vessel alteration has included capillary obliteration, thickening of arterial walls and fragmentation of elastica in larger vessels.

In general, these alterations have been reproduced in experimentally irradiated rodents (Jennings and Arden, 1961, 1962) but refined understanding of the precise sequence of events has been hampered by several clinical and experimental difficulties: 1) limitations of resolution of light microscope; 2) superimposed infection (Kurohara and Casarett, 1972); 3) species variation; 4) influence of damage of remote tissues in whole body radiation (as suggested by experiments with parabiotics [Goldenberg et al., 1968]); 5) influence of

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uneven ventilation and variations in O_2 tension; and 6) in humans, underlying coexisting pulmonary disease.

Elegant electron micrographic studies have more recently emphasized primary vascular endothelial injury (Phillips, 1966; Phillips, 1973; Adamson et al., 1970). The description that follows is derived primarily from Adamson et al.: Within the first week after 600 rad total body irradiation, vacuolation of capillary endothelial cells occurs with ballooning of cytoplasm into the capillary lumen. Subendothelial swelling separates the endothelial cell from the supporting basement membrane. The epithelium of Type I squamous and Type II cells with lamellar bodies remains relatively unaffected. The endothelial vacuolation so stretches the cytoplasm that capillary lumen become occluded, and by ten days the basement membrane may be completely stripped of the overlying endothelium. Platelet thrombi attach to the denuded basement membrane by two weeks. At this time minor alterations in epithelial cells may be appreciated. Fibrin is deposited within the platelet thrombus, and organization with fibroblasts and subsequent deposition of collagen follows.

Biochemistry of Fibrosis. Collagen Chemistry and Crosslink Formation
Research concerning the biosynthesis of scar has centered on collagen metabolism since it is the principal extracellular protein synthesized during active fibrosis and since collagen fibers are the predominant material in scar (Ramachandran, 1967). The maturation of collagen involves the progressive loss of solubility by extensive crosslink formation. It is appropriate to review fundamental collagen chemistry and the mechanism of crosslink formation (Traub and Piez, 1971; Gallop et al., 1972).

The molecule is an asymmetric rigid rod with dimensions of 15 Å x 3000 Å. Each molecule consists of three polypeptide chains each of which contains approximately 1,000 amino acid residues. Throughout the course of most of the molecule, polypeptide strands are arranged in the unique collagen helix pattern. In each polypeptide chain every third residue is glycine. These glycyl residues occupy the axial plane. Individual collagen molecules form fibrils by precipitating in a linear quarter-stagger array. Fibril formation proceeds spontaneously in the extracellular space under physiologic conditions. Crosslink formation is an important element in fibril stabilization. Crosslinks exist within each molecule between the polypeptide chains in the non-helical amino terminal portion. Intermolecular crosslinks within the fibril are much more extensive but somewhat less well understood. These crosslinks link many single molecules into a large polymer. The detailed chemistry of collagen

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crosslinking has been well studied in organs such as skin and bone. The ϵ -amino group of specific lysyl residues, predominantly those in the amino-terminal region, undergo enzymatic oxidative deamination (allysine oxidase) to the ϵ -amino adipic- δ -semialdehyde. Crosslinks are formed when modified residues on adjacent chains undergo aldol condensation reactions or when Schiff base formation takes place between a modified lysyl or hydroxylysyl residue and one which has not been modified^{or} with another amine. These Schiff bases become stabilized by subsequent reduction. More recently a greater variety of additional spontaneous condensations of aldehydes has been identified. The products of these condensations have been isolated from highly crosslinked collagen polymers and suggest the involvement within one complex crosslink of three lysines and one histidine from four separate polypeptide strands. These crosslinks may occur in the helical portion of the molecule, but their precise location is as yet unknown. The key initial reaction for the formation of all crosslinks, both intra- and inter-molecular, involves the initial oxidative deamination of the ϵ -amino group of lysine. This enzyme, allysine oxidase, may be selectively inhibited by lathrogens. Thus crosslink formation can be blocked without affecting collagen synthesis, collagen secretion or spontaneous fibril formation. The paradigm compound β -aminopropionitrile (β APN) has been demonstrated in vitro specifically to inhibit the enzyme allysine oxidase (Pinnell and Martin, 1968; Siegel et al., 1970; Siegel and Martin, 1973). Chronic exposure of animals during growth to this compound results in connective tissue of low tensile strength and high solubility.

Biochemical Modification of Scar Formation

Because of the central importance of collagen synthesis and crosslinking in fibrosis, scar formation and wound healing, there is wide interest in investigating compounds which may potentially manipulate collagen metabolism. The most extensively studied method of altering collagen is the use of lathrogens such as β APN, and attempts have been made to use lathrogens to ameliorate the functional alteration associated with fibrosis in experimental animals and in human disease. Models in which early evidence of probable effectiveness have been obtained include silica-induced pulmonary fibrosis (Leveue and Byc, 1964), carbon tetrachloride-induced hepatic fibrosis (Fiume, 1962), and lye-induced esophageal stricture (Davis, 1971). Pilot clinical studies have not been as rewarding. The hide-bound skin of scleroderma has not been improved by the use of lathrogens (Keiser and Sjoerdsma, 1967), and while increased range of

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motion may be affected by lathrogens following tendon repair, this beneficial effect has been lost when the drug is stopped (Peacock et al., 1968).

A second method for the inhibition of collagen crosslinking has utilized compounds which inhibit crosslinking by complexing with the aldehydes that are formed from allysine oxidase. D-Penicillamine is the model compound which has been used clinically (Harris and Sjoerdsma, 1966). Patients with systemic sclerosis have been treated with relatively disappointing results. They may have slightly increased mobility of skin by special testing, but the progression of disease is unaffected (Fulghum and Katz, 1968).

A more recent major investigational pathway is the use of proline analogs such as azetidine-2-carboxylic acid, 3-4-dehydroproline, cis-hydroxyproline and 4,5-dehydrolysine (Lane et al., 1971; Rosenbloom and Prockop, 1970). Effects on collagen synthesis with all these compounds have been recently investigated. The first two of these compounds are incorporated into the collagen molecule on the order of four residues per 1,000. They apparently inhibit extrusion of the collagen molecule from the cell so that formation of fibrils is diminished. Some experiments indicate that these compounds decrease granuloma formation by as much as 50% when tested in the carrageenin granuloma model.

The potential role of corticosteroids in the management of radiation lung injury is as yet unresolved. In patients acute pneumonitis may respond to corticosteroids (Cosgriff and Kligerman, 1951; Friedenberg and Rubenfeld, 1954; Ruben et al., 1958), and flares of acute radiation pneumonitis on occasion appear to be recalled following withdrawal of corticosteroids which have been incidentally administered (personal observation). It is unclear, however, whether corticosteroids will affect the development of fibrosis. Studies with animal models have been inconclusive (Brown, 1956) to a large extent because of infection (Stewart, personal communication).

Biochemistry of Lung Collagen

Very little published information exists which specifically concerns lung collagen despite its obvious importance both in the normal structure of the lung and in the diseases in which a fibrosis reaction is prominent. Anatomic considerations lead to the prediction of at least three kinds of collagen: 1) collagen fibrils contribute to the interstitial matrix; 2) collagen of the cartilaginous type (Miller, 1972) is present in the bronchial skeleton; 3) the alveolar and capillary basement membrane may also be predominantly a collagen with a unique structural gene analogous to basement membrane of renal glomerulus and

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and lens capsule (Kefalides and Denduchis, 1969). None of these collagens has been individually, cleanly isolated, and no primary structural data is as yet available. In addition, it is not known whether the collagen seen in a pathologic fibrosis reaction shares the structural gene of the involved organ. There are no studies which effectively deal with spontaneous rates of collagen turnover in the lung, and none which quantify collagen synthesis during periods of active fibrosis in the lung.

The specific goals of this study may be summarized as follows:

1. Characterization and comparison of lung collagens and the collagen of fibrosis
 - a. — Isolation by chromatography
 - b. Cyanogen bromide peptide maps and amino acid analysis
2. Evaluation of therapeutic intervention in lung fibrosis
 - a. Lathrogen (BAPN)
 - b. Corticosteroid
3. Refinement of the model of lung fibrosis following radiation injury
 - a. Improvement of reproducibility by attention to bacteriologic control
 - b. Development of a reliable and sensitive biochemical measure of pathologic fibrosis
 - c. Detailed correlation of morphologic, biochemical and functional alterations

Methods to be utilized include:

1. Techniques

Radiation (supervised by Dr. J. Martin Brown)

Anesthetized mice (sodium pentobarbital, 67.5 mg/kg IP) will be placed in a jig which allows irradiation of six animals simultaneously. The radiation field will be right hemithorax alone or entire thorax with midline shielding to protect the esophagus. Conditions will be 250 kv, 15 ma, focused skin distance 40.5 cm, added filtration of 0.25 mm Cu and 1.0 mm Al, HVL 1.1 mm Cu and a dose rate of 157 rads. All measurements are made with a Philips dosimeter calibrated against a standard Victoreen chamber (NBS calibrated). A factor of 0.95 is used to convert exposure in roentgens to rads in tissue.

Bacteriologic Control and Animal Selection (supervised by Dr. J. Martin Brown)

The animals used in these experiments will be specific pathogen free (SPF). In several reported studies (Brown, 1956; Kurohara and Casarett, 1973) as well as in our own preliminary work, microscopic changes are characterized by great

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variation among animals in both acute inflammatory changes as well as degree and distribution of fibrosis. It appears that an important variable may be infection. Our preliminary studies have shown the LD_{50/160} for thorax-irradiated conventional mice to be approximately 1400 rads single dose. The comparable statistics elsewhere (Yuhás, 1973) is twice this dose when SPF mice are utilized. We are in the process of building up stocks of SPF mice (C3H/Km, Balb/C, and C56BL/Ka). The procedure involves caesarian delivery under sterile conditions and foster nursing on SPF mice as described (Serrano, 1971). Strict barrier conditions will be maintained for breeders. Animals used in experiments will be kept under less rigid conditions in cages with filter tops (Maryland Plastics, New York). All operations such as drug injections and irradiation will be performed under sterile conditions in filtered, laminar flow air.

Microscopic Evaluation (supervised by Dr. Luis Fajardo)

Dose/response curves for pathologic changes in morphology will be performed on unilaterally irradiated mice to avoid the systematic selection of minimally affected animals which results from studying survivors of whole thorax irradiation. Mice will be treated at age 4 to 6 months. In initial studies mice will be sacrificed by decapitation to minimize anesthetic induced changes. Lungs will be fixed in 10% buffered formalin and sections 4-6 μ thick will be stained by hematoxylin and eosin as well as Gamori's trichrome method for connective tissue. In initial experiments the dose delivered will range from 600 to 3600 rads in a single exposure, and animals will be sacrificed at 2, 4, 8, 16, and 32 weeks. Scoring will include assessment of presence and distribution of both inflammatory changes (granulocytes, histiocytes, lymphocytes, fibrin) and fibrosis. Conditions of bacteriologic control and irradiation will be varied to minimize infection and also death by radiation damage to other organs such as esophagus, heart and pericardium.

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Biochemical Measures of Degree of Fibrosis (supervised by Dr. John Daniels)

Two approaches to the biochemical quantification of the collagen of lung fibrosis will be pursued:

- a) Entire lungs from unilaterally irradiated mice will be separated, hydrolysed (6 N HCl, anaerobic), and hydroxyproline content determined by Dowex 50 separation and ninhydrin reaction on an automatic amino acid analyser. Preliminary studies have shown this method feasible with a standard deviation of about 10%.
- b) Highly specific collagenase will be prepared by gel permeation chromatography and selective inhibition with N-ethyl maleimide (Peterkofsky and Diegelmann,

1971). Powders of irradiated lungs will be prepared in a Wiley mill, washed in neutral 20% NaCl and 0.5 M acetic acid and incubated with collagenase. Solubilized material will be hydrolysed and amino acid hydrolysis performed both to quantitate material released and to determine, independent of hydroxyproline, that it is collagen. Since the degree of hydroxylation of collagen is an unpredictable variable, this second method will independently assess the validity of the easier hydroxyproline determinations.

Characterization of Lung Collagen

Lung collagen of mature mice (age 4 to 6 months) will be solubilized by limited proteolysis with pepsin (Kefalides and Denduchis, 1969; Miller, 1972; Daniels, 1973). In parallel studies weanling mice will be raised on a lathyritic diet (see below) and lungs sequentially extracted with 1 M NaCl, pH 7.4 and 0.5 M acetic acid. The collagen will be purified by salt precipitation (Bornstein and Piez, 1966). After denaturation, monomers (α -chains) will be selected by gel permeation chromatography (agarose, BioGel A 5) and separated by ion exchange chromatography sequentially on columns of carboxymethyl cellulose and diethylaminoethyl cellulose. Isolated α -chains will be characterized by amino acid analysis and by peptide maps developed after limited hydrolysis at methionyl residues by cyanogen bromide (CNBr) (Bornstein and Piez, 1965; Butler et al., 1967).

The collagen of the fibrosis reaction will be obtained by placing adult mice on lathyritic diets immediately after maximally effective hemithoracic irradiation and isolating collagen without pepsin digestion. Under these conditions we would expect to be able to extract only the collagen synthesized after exposure to radiation (while on BAPN) and thus separate the collagen of the fibrosis reaction from that pre-existing in normal lung. Collagen thus obtained will be characterized as before (chromatography, amino acid analysis and peptide maps) and compared with fractions isolated from normal lung.

Administration of BAPN

All mice will be maintained on Purina Cat Chow. This feed is in the form of a star-shaped kernel with a high surface volume ratio. BAPN will be dissolved in water and sprayed on the kernels (2 grams BAPN/kg food) which are air-dried. Feasibility studies have confirmed that this effectively produces lathyrism in growing C3H mice and is without apparent toxicity over a six-month period for adult (4 to 6 months old) mice.

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Measurement of Pulmonary Compliance

It is expected that the fibrosis reaction will be associated with decreased compliance. Mice to be studied will be sacrificed by CO₂ intoxication. The trachea will be percutaneously cannulated with a plastic catheter which is secured by a ligature. After intubation the thoracic cage is dissected free by removing overlying skin and transecting at the level of the neck and the diaphragm. The diaphragm is removed. In feasibility studies, we were able to obtain such preparations consistently and without air leaks from both normal animals and mice after irradiation. Compliance is measured by connecting the cannula to a pressure transducer and to a calibrated syringe. Air is added or withdrawn in 0.1 ml increments, and the intrapulmonary pressure is electronically recorded.

2. Summary of Anticipated Sequence of Experiments

- a. Group 1. Dose/response for morphologic change with hemithoracic irradiation.
Dose/response for survival. Bilateral irradiation. Establishment of LD_{50/160} in SPF C3H mice.
- b. Group 2. Methodology for biochemical measure. Comparison of time-dependent change between irradiated and shielded hemithorax and selection of method (hydroxyproline vs. collagenase release).
Characterization of collagen of fibrosis reaction (hemithoracic irradiation and lathyritic diet).
Compliance studies in sublethally, bilaterally irradiated animals.
- c. Group 3. Correlative studies: morphologic, biochemical and mechanical changes as a function of time and dose after bilateral irradiation.
Effects of BAPN on correlative studies and on LD_{50/160} days.

General Significance of Proposed Study

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This injury model if successfully developed will have a general utility in future studies. Studies of other effectors of lung injury including the entire range of chemical inhalents could be extended to utilize the biochemical and functional techniques explored here. A model will be established which evaluates the effects of therapies in functional and biochemical as well as morphologic dimensions. In short, the model system may provide a general experimental

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tool for diverse problems in the etiology and treatment of pulmonary fibrosis.

Lung collagen will be characterized in the way other collagens have been characterized, and the general question of whether the structural gene for fibrosis is the same as the gene for interstitial collagen of the affected organ will be approached.

The specific question of usefulness of lathrogens in ameliorating the functional disturbances secondary to scar formation in radiation injury of the lung will be answered. This may have important implications for this same question in other tissues and following other injuring agents.

References

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Daniels, J.R., in preparation.
Fajardo, L.F., and J.R. Stewart, *Am. J. Path.* 59:299, 1970.
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- Stewart, J.R., K.E. Cohn, L.F. Fajardo, et al., *Radiol.* 89:302, 1967.
- Traub, W., and K.A. Piez, *Adv. Prot. Chem.* 25:243, 1971.
- Yuhas, J. *Nat. Cancer Inst.* in press, 1973.

Facilities Available

Personal laboratory space is 400 square feet of standard design. It is generally well equipped and well stocked. Common equipment facilities and controlled temperature rooms are in adjacent space. Personal and common equipment capabilities include extensive column chromatographic equipment, spectrophotometer, recorder, pH meter, flash evaporator, CO₂ incubator, laminar flow hood, preparative and analytical ultracentrifuges, scintillation counters, amino acid analyser, high voltage electrophoresis, acrylamide gel electrophoresis, sonifier and a variety of partition chromatographic apparatus. Animals will be housed (as noted in Methods) in a new barrier facility under strict bacteriological control.

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Biographical Sketch of Principal Investigator

John R. Daniels

Date and Place of Birth:**REDACTED**Education:

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A.B. Stanford University
M.D. Stanford UniversityResearch and/or Professional Experience

- 1972- Assistant Professor, Department of Medicine, Division of Oncology,
Stanford University School of Medicine
- 1970-72 Instructor, Department of Medicine, Division of Oncology, Stan-
ford University School of Medicine
- 1969-70 Senior Resident, Department of Medicine, Stanford University
School of Medicine
- 1966-69 Research Associate, National Institute for Dental Research,
National Institutes of Health, Bethesda, Maryland
- 1965-66 Assistant Resident, Department of Medicine, Stanford University
School of Medicine
- 1964-65 Intern, Department of Medicine, Stanford University School of
Medicine
- 1964 Post-doctoral Fellow, Department of Cell Biology, Albert Einstein
College of Medicine, New York
- 1959-62 Trainee, Department of Pharmacology, Stanford University School
of Medicine

Major Investigative Interest:

Connective Tissue Chemistry; Clinical Oncology

Honors:

Diplomate, American Board of Internal Medicine, 1972

Research Support

Current Support:

Mammalian Collagenase and Basement Membrane Collagen, NIH #AM 14896-03.
February 1, 1973 - January 31, 1974. \$26,117. 50% effort.

Pending applications:

Cancer and Bone Metabolism, American Cancer Society. January 1, 1974 -
December 31, 1976. \$132,903. 15% effort.This proposal is also being submitted to the NIH as part of a Program
Project Grant entitled Laboratory Studies: Support of Clinical Cancer
Center. January 1, 1974 - December 31, 1976. \$700,245. 23% effort.PublicationsKalman, S.M., and J.R. Daniels, Effect of injected estradiol on the uptake of
 α -aminoisobutyric acid by tissues of the ovariectomized rat. Biochem.
Pharm. 8:250, 1961.

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Publications (continued)

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- Lazarus, G.S., J.R. Daniels, J. Lian, and M.C. Burleigh, Role of granulocyte collagenase in collagen degradation. *Am. J. Path.* 68:565, 1972.
- Daniels, J.R., J.M. Lian, and G.S. Lazarus, Human granulocyte collagenase. *J. Biol. Chem.*, in press.

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Biographical Sketch of Co-Investigator

John Martin Brown

Date and Place of Birth:**REDACTED**Education:

B.Sc. Birmingham University, Birmingham, England
 M.Sc. London University, London
 D.Phil. Oxford University, Oxford

Research and/or Professional Experience:

1971- Assistant Professor, Department of Radiology, Radiobiology Research Division, Stanford University School of Medicine
 1970-71 Research Associate, Department of Radiology, Radiobiology Research Division, Stanford University School of Medicine
 1968-70 Post-doctoral Fellow, Department of Radiology, Radiobiology Research Division, Stanford University School of Medicine

Major Investigative Interest:

Mammalian cell, tissue and tumor radiobiology, cell kinetics, radiation carcinogenesis

Honors:

Medical Research Council Scholarship, Oxford University, 1965-68
 NIH Fellowship in Radiobiology and Cancer Research, 1968-70
 American Cancer Society (California Division) Dernham Senior Fellowship in Oncology, 1971-74

Research Support:Current Support:

Dernham Senior Fellowship in Oncology, #D-188, American Cancer Society (California Division). September 1, 1972 - August 31, 1973. \$16,400. Salary support only.

Training Program in Cancer Research in Radiology, NIH #CA-05008, NIH-NCI. July 1, 1972 - June 30, 1976. \$311,990. 15% salary support.

Pending support:

CORE Grant, NIH #CA-10372-07, NIH-NCI. September 1, 1973 - August 31, 1974 \$436,771. 10% salary support.

Dr. Brown is also named on the Program Project Grant of this project referred to on Dr. Daniels' Biographical Sketch. 10% salary support.

Publications:

Brown, J.M., and R.J. Berry, The relationship between diurnal variation of the number of cells in mitosis and of the number of cells synthesizing DNA in the epithelium of hamster cheek pouch. Cell Tissue Kinet. 1:23, 1968.
 Brown, J.M., and R.J. Oliver, A new method of estimating the cell cycle time in epithelial tissues of long generation time. Cell Tissue Kinet. 1:11, 1968.

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Publications (continued)

- Brown, J.M., and R.J. Berry, Effects of X-irradiation on cell proliferation in normal epithelium and in tumors of the hamster cheek pouch. In Effects of Radiation on Cellular Proliferation and Differentiation, IAEA Symposium, pp. 475-491, 1968.
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- Brown, J.M., and D. Goffinet, A technique for intra-arterial infusion of tumor-bearing mice. J. Lab. Clin. Med. 76:175, 1970.
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Biographical Sketch of Co-Investigator

Luis F. Fajardo

Date and Place of Birth:**REDACTED**Education:**REDACTED**

A.B. Liceo de la Salle, Bogota, Colombia

M.D. Universidad Nacional, Bogota, Colombia

Research and/or Professional Experience:

- 1972- Associate Professor, Department of Pathology, Stanford University School of Medicine
- 1966-72 Assistant Professor, Department of Pathology, Stanford University School of Medicine
- 1965- Pathologist, Veterans Administration Hospital, Palo Alto, California
- 1962-65 Chief, Department of Pathology, Universidad Nacional, Bogota, Colombia
- 1960-62 Assistant Professor, Department of Pathology, Universidad Nacional, Bogota, Colombia
- 1959-60 Pathologist, New Britain General Hospital, New Britain, Connecticut

Major Investigative Interest:

Radiation pathology

Honors:

Founder, First Volunteer Blood Bank in Colombia, 1961
 Founding Member and Secretary-Treasurer (1963-65), Colombian Society of Pathology

Research Support:

Current Support:

Sequential Study of Ultrastructure Changes in Experimental Radiation Heart Disease, MRIS #2735-01, VA Research Funds. July 1, 1972 - June 30, 1975
 \$68,400. 25% effort.

Publications

- Cohn, K.E., J.R. Stewart, L.F. Fajardo, and W. Hancock, Heart disease following radiation. *Medicine* 48:281, 1967.
- Stewart, J.R., K.E. Cohn, L.F. Fajardo, W. Hancock, and H.S. Kaplan, Radiation-induced heart disease. A study of twenty-five patients. *Radiol.* 89:302, 1967.
- Stewart, J.R., L.F. Fajardo, K.E. Cohn, and V. Page, Experimental radiation-induced heart disease in rabbits. *Radiol.* 91:814, 1968.
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Publications (continued)

- Stewart, J.R., and L.F. Fajardo, Dose response in human and experimental radiation-induced heart disease. Application of NSD concept. Radiol. 99:405, 1971.
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- Stewart, J.R., and L.F. Fajardo, Radiation-induced heart disease. In J. Vaeth (ed.) Frontiers of Radiation Therapy and Oncology, Vol. 6, S. Karger AG, Basel, pp. 274-288, 1972.
- Fajardo, L.F., and H. Sarasti, Technique for percutaneous needle biopsy of bone and marrow. Calif. Med. 177:21, 1972.
- Rockwell, S.C., R. Kallman, and L.F. Fajardo, Characteristics of a serially transplanted mouse mammary tumor and its tissue culture adapted derivative. J. Nat. Cancer Inst. 49:735, 1972.

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#015B - ESTERLY

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

July 31, 1973

Grant application #815B
CHRONIC PULMONARY DISEASE

To: The committee comprising Drs. Loosli, Sommers and Wyatt

Subject: John R. Esterly, M.D., University of Chicago, Illinois
Continuation application No. 815B
"Resolution of Pulmonary Injury"

History

This investigation has been supported by CTR since 1971.
The 1972 - 1973 grant was in the amount of \$30,028., without assurance
of further support.

Application #815B requests \$34,482. Although presented on
renewal forms, this is a "continuation" request which competes as a
new request, without commitment.

Documents Submitted (attached)

1. Application dated July 20, 1973.
2. Progress Report (4 pages).
3. "Experimental Bacterial Pneumonia . . ." Pine, Richter,
and Esterly, with original micrographs and electron-
micrographs. (In press, Amer. J. Path.)

FWN:gh


F.W.N.

Attachments

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Drs. Loosli
Sommers
Wyatt

CHRONIC PULMONARY DISEASE

#815B

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 50TH STREET
NEW YORK, N. Y. 10022

Application For Renewal of Research Grant

First ☐ Second ☒

Date: July 20, 1973

1. Name of Investigator(s): (include title and degrees)

Principle Investigator - John R. Esterly, M.D., Associate Prof., Dept. of Pathology

Co-Investigator - Ward R. Richter, D.V.M., Prof. of Pathology and Director, A.I.C Facility.

2. Institution &

Address:

University of Chicago
950 East 59th Street
Chicago, Illinois 60637

3. Short Title of Project: Resolution of Pulmonary Injury

4. Proposed Renewal Starting Date: (Anniversary or other) October 1, 1973

5. Discuss any Important Changes or Additions to Objectives or Specific Aims: See Progress Report

6. Give a Brief Statement of your Working Hypothesis if altered or modified:

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7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

8. Additional Requirements:

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

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Publications or Papers in Press resulting from the Project or closely related work

Bacterial Pneumonia: Ultrastructural and Histochemical Observations. Janet H. Pine, Ward R. Richter and John R. Esterly. Amer. J. Path. Vol. 70, pp. 46A, 1973. (Abstract of presented paper). The whole manuscript is in press in Amer. J. Path.

11. Budget (for coming year)

A. Salaries (Personnel by names or category)

Professional

John R. Esterly, M.D.

% time

30%

Amount

0

Ward R. Richter, D.V.M.

10%

0

Technical

* Research Technician

50%

4150

* David Baron, EM Technician

100%

8500

Student Technician

50%

3120

* Fringe benefits @ 13.5%

1708

Sub-Total

B. Consumable Supplies (list by categories)

Breakdown for supplies on following page

17478

Sub-Total

C. Other Expenses (itemize)

2 trips to attend National Meetings
(1 East Coast, 1 West Coast)

10306

600

Photographs

600

40% service contract on RCA-EMU_{4B}

500

Publications

500

Sub-Total

D. Permanent Equipment (itemize)

None

2200

0

E. Overhead (15% of A + B + C)

4498

Total

34482

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's Statement of Policy Concerning Conditions and Terms Under Which Project Grants Are Made.

Signature

Director Project

1.

Signature

Business Officer of the Institution

Telephone

Telephone

L.C.W. Library, 4949 J. R. M. St. S.E. 11111 Telephone

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Consumable Supplies

Purchase of diamond knife	645
Sharpening of diamond knife	250
Purchase: Specified strain of rats	
300/yr. @ 3.50 ea.	1050
Care @ A.J.C. @ .049/rat X 13,500 rat days	661
Histochemical substrates	2700
Chemicals & histological supplies	3000
EM photographic supplies & chemicals	1200
Glassware & small instruments	800
	<u>10306</u>

Comment on Budget Changes. The differences between this budget and those for the previous years reflects the increase emphasis (supplies and labor) on electron microscopy and the inclusion of publication costs, e.g. the article in press will cost nearly \$500 for page & illustration changes. The majority of the increase, however, over that originally projected, is due to increased wage rates.

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Digestive Enzymes in the Lung (Renewal)	Tuberculosis Institute of Chicago and Cook County	11,980.00	7-1-73/6-30-74
Research Career Development Award	National Heart and Lung Institute	25,000.00	7-1-71/6-30-75
Resolution of Pulmonary Injury	Council for Tobacco Research	29,280.00 .	7-1-72/9-30-73

Pending

1003539556

#921 - FINLEY

1003539557

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

July 17, 1973

Grant Application No. 921

To: The committee comprising Drs. Gardner, Loosli and Wyatt

Subject: Theodore N. Finley, M.D., Mount Zion Hospital, San Francisco
New application No. 921
"Studies on Bronchopulmonary Lavage Fluid in Smokers and
Patients with Respiratory Diseases."

History

Dr. Gardner visited Dr. Finley on July 12. The proposal discussed then was subsequently shortened from five to three years.

Application No. 921 requests \$90,712 plus two additional years.

Document Submitted

Attached is application dated July 12, 1973 (19 pages).

Comment

Dr. Finley states that Dr. Gary Huber will write in support of this application. Dr. Huber's comment will be forwarded to you as soon as received.

F.W.N.
F.W.N.

FWN:wg
Encl.

1003539558

Comm.

Dr. Gardner

Dr. Loosli

Dr. Wyatt

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

110 E 59th STREET
NEW YORK, NEW YORK 10022

JUL 16 1973

APPLICATION FOR RESEARCH GRANT

Date: July 12, 1973

1. Name of Investigator(s): (include Title and Degree)

Theodore N. Finley, M.D. Director, Pulmonary Laboratory
W. Lawrence Drew, M.D., Ph.D. Director, Microbiology
David W. Golde, M.D. Assistant Professor of Medicine

2. Institution & Address:

Mount Zion Hospital and Medical Center, 1600 Divisadero Street
San Francisco, California 94115

3. Short Title of Project:

Studies on Bronchopulmonary Lavage Fluid in Smokers and Patients with
Respiratory Diseases.

4. Proposed Starting Date:

December 1, 1973

5. Anticipated Duration of this Specific Study:

Three (3) years to December 31, 1976

6. Brief Description of Objectives or Specific Aims:

(see attached sheets)

7. Give a Brief Statement of your Working Hypothesis:

(see attached sheets)

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

(see attached sheets)

9. Physical Facilities Available (Where Other than Administering Organization
Indicate Geographical Location).

(see attached sheets)

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

(see attached sheets)

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Introduction: Bronchopulmonary lavage in human subjects offers a unique opportunity to obtain and study alveolar macrophages and to diagnose certain pulmonary diseases. We have pioneered this technique and have lavaged approximately 300 humans.

The human alveolar macrophage represents the first line of cellular defense for the lung. Although relatively little is known about the functional and proliferative properties of this cell, it appears to be significantly involved in various pulmonary disease states. We propose to study, by newly developed *in vitro* techniques, cells obtained at bronchopulmonary lavage in both normal human subjects (smokers and nonsmokers) and in patients with various clinical respiratory diseases. These studies are designed to provide information on: 1) the type and quantity of cells present in the lavage fluid, 2) their proliferative capacity, 3) their ability to mature, 4) their functional properties 5) the mechanisms involved in particle ingestion and microbial killing, and 6) their morphologic characteristics. Infectious processes may be pathogenically responsible for many of the responses that have been attributed to tobacco smoking. Therefore, the following additional studies will be performed on all lavage specimens: 1) viral, bacterial, fungal, mycoplasma and mycobacterial cultures, 2) cytology and 3) special stains for pneumocystis as well as fungi, AFB and other organisms. The morphology of the alveolar macrophage will be examined at both the light and electron microscopic level and correlated with clinical data and functional studies.

Experimental Design:

1. Determination of the proliferative capacity of the alveolar macrophage in normals, cigarette smokers and in patients with various respiratory diseases.

We have developed a new technique which permits the cultivation of human hematopoietic cells in liquid suspension. This method involves the use of an *in vitro* diffusion chamber apparatus which enables the cultivation of cells in liquid suspension and their easy retrieval for periods of up to one month. This technique is particularly well suited for studying cells of the macrophage series and has proved successful in culturing human pulmonary lavage alveolar macrophages obtained to date from four subjects. Our results so far indicate that cigarette smokers have a several fold greater number of recoverable alveolar macrophages than non-smokers. Data obtained in these initial studies also indicate that these cells have proliferative capacity. The significance of this must be clarified as a high priority.

The macrophages will be obtained by bronchopulmonary lavage using procedures previously described. The cells are cultured in the special chambers and harvested at periods ranging up to 30 days. Proliferation is assessed by changes in viable cell counts at various intervals and by the use of three techniques of tritiated thymidine radioautography. The first method involves a pulse label with radiothymidine one hour prior to harvest. This provides information concerning the cells in DNA synthesis at the time of sacrificing the culture. In the second method the cells are exposed to tritiated thymidine for one hour after isolation from the lavage fluid.

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They are then cultured in medium containing "cold thymidine" and the radioautographic distribution of label is followed in culture. This method allows for confirmation of replication and provides data on progeny-progenitor relationships and *in vitro* cell maturation. The third technique involves culturing cells continuously in the presence of tritiated thymidine for periods up to one week. In this method all cells passing through S phase will take up the label and thereby permit an estimation of proliferative potential in cell populations with a small growth fraction. Clearly, thymidine incorporation is not synonymous with proliferation in that DNA repair can occur without cellular replication. Therefore, in addition to total viable cell counts, mitotic indices will be determined in cultures containing colchemid. This information will be correlated with the radioautographic data. Labelled mitoses will be looked for in cultures containing both colchemid and tritiated thymidine.

Our published work demonstrates that pulmonary alveolar macrophages recovered from normal smokers have the ability to produce a factor that stimulates bone marrow cells to proliferate. This is of crucial importance for further study, in that the bone marrow is the most widely accepted origin of the alveolar macrophage. The production of a marrow stimulating factor, the increased number of recoverable alveolar macrophages and the increased number of circulating white blood cells in smokers appear to all be potentially related. The significance of this, in terms of health or disease, needs clarification.

2. Determination of the capacity for phagocytosis and microbial killing of cells obtained by bronchopulmonary lavage in smokers and non-smokers.

Green, working with experimental animals *in vitro*, has implied that alveolar macrophage function can be impaired directly by tobacco cigarette smoke in a dose-dependent manner. This observation could not be confirmed in alveolar macrophages recovered from normal human cigarette smokers, in studies published by Harris and Swenson. These discrepancies must be clarified. Huber has described the development of adaptive tolerance in animals intermittently exposed to environmental oxidants. Potential differences in the ability of the host to develop tolerance to tobacco smoke and the possible role these differences might have in the pathogenesis of lung disease need to be studied.

Cells obtained at lavage from smokers and nonsmokers will be studied to determine their phagocytic capacity using various microorganisms and particles, including carbon and latex. The process of phagocytosis will be documented by phase, interference, and electron microscopy and, in selected instances, cine-photomicroscopy on living cells. Microbial killing by phagocytic cells will be assessed by the standard colony count technique. In addition, a new method developed by Dr. Martin J. Cline, which permits assessment of microbial killing by various cell classes using a radioautographic technique, will be utilized. Similar tests will be performed on cells cultured at various intervals in order to determine changes in phagocytic and microbial killing capacity in the *in vitro* environment and to serve as a functional marker for cellular maturation. Results obtained in normals will be compared to those noted in various pathologic conditions.

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3. To determine the effect of surface active materials on the capacity of pulmonary macrophages for phagocytosis and microbial killing.

We have previously described quantitative differences in pulmonary surface active materials in smokers and nonsmokers. Synthesis of dipalmitoyl lecithin (the primary surfactant) by alveolar macrophages and the dependency of macrophages on the presence of a surfactant fraction required for intracellular killing have also been reported. It is of crucial importance to investigate these interrelationships in smokers and nonsmokers who are normal and who have disease.

The previously outlined tests for phagocytosis and microbial killing will be performed in the presence of various surface active constituents obtained by pulmonary lavage. The ability of the cells to ingest particles and to kill microorganisms will be studied in materials obtained from lavage in normals, smokers and patients with other pulmonary pathology. In addition, the participation of lipids will be assessed by using radioactively labelled purified lipids and determining the degree of cell entry in relationship to phagocytosed particles by radioautography.

4. To determine the usefulness of alveolar macrophages to diagnose viral illnesses, particularly in smokers. The potential role of viruses as etiologic factors in many of the lung diseases commonly attributed to tobacco smoking has been, for the most part, excluded.

Lavage macrophages from smokers and nonsmokers, with and without disease, will be cultured and studied with fluorescent antibodies for viral respiratory pathogens. Also direct hemadsorption and hemadsorption inhibition for identification will be attempted. In addition to diagnostic aspects, the lavaged cells from patients in several categories (normal, smokers, infected immunosuppressed, etc.) will be studied for their response to specific viral antigens as measured by transformation and interferon production.

Significance of this Research:

The results obtained from studies outlined in this proposal will provide applied information on the functional characteristics of the cells found in the bronchopulmonary spaces in smokers and nonsmokers. These data can shed light on the participation of the alveolar macrophage and the underlying pathogenetic mechanisms in various respiratory disease states. These studies are of key importance in sorting out the etiologic factors in many respiratory diseases and will help significantly to clarify many of the currently existing controversies on tobacco and health.

The effect of cigarette smoking on the pulmonary alveolar macrophage will be looked at intensively and resultant information on the reaction of the normal lung to smoke inhalation, and consequences of these reactive phenomena, can be used to resolve this important public health question. Information obtained on the proliferative, maturational, and functional properties of the human pulmonary alveolar macrophage will ultimately provide a means for favorably affecting the pulmonary response in various pathologic conditions.

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Use of Human Subjects:

Full approval for bronchopulmonary lavage in consenting adults has been obtained by the Research Sub-Committee on Clinical Research and Investigation Involving Human Subjects at the Mount Zion Hospital and Medical Center.

Physical Facilities Available: (Where other than Administering Organization indicate geographical location).

The bronchopulmonary lavages will be performed by Dr. Theodore N. Finley at Mount Zion Hospital and Medical Center in San Francisco. Dr. Finley has extensive experience with this technique and his department is fully equipped for the procedure. In vitro cellular studies will be performed in the laboratories of the Cancer Research Institute of the University of California in San Francisco. Currently, all of the techniques described in this application are being performed in these laboratories. The Microbiologic cultures including virologic will be performed in the Clinical Laboratories of Mount Zion Hospital and Medical Center which includes a functioning clinical virology laboratory under the direction of Dr. W. Lawrence Drew. Interferon and cell transformation studies will be performed in the space described below; these techniques are also in use at present.

Space available encompasses 1400 sq.ft. at Mount Zion Hospital. The laboratory is well equipped for tissue culture work with two tissue culture rooms, laminar flow hood, incubators and a wide range of special microscopic equipment including cine-photomicroscopy. Electron microscopy time is purchased under contract from the Department of Pathology at the University of California Medical Center, San Francisco.

1003539563

Theodore N. Finley, M.D.

12. Publications:

1. Golde, D.W. and Cline, M.J. Growth of Human Bone Marrow in Liquid Culture. Blood Jan. 1973.
2. Pratt, S.A., Finley, T.N., Smith, M.H. and Ladman, A.J. A Comparison of Alveolar Macrophages and Pulmonary Surfactant obtained from the Lungs of Human Smokers and Nonsmokers by Endo-bronchial Lavage. Anat. Record 163: 497, 1969.
3. Pratt, S.A., Smith, M.H., Ladman, A.J. and Finley, T.N. The Ultra-structure of Alveolar Macrophages from Human Cigarette Smokers and Nonsmokers. Lab. Invest. 24:5, 331, 1971.
4. Finley, T.N. and Ladman, A.J. Low Yield of Pulmonary Surfactant in Cigarette Smokers. New Eng.J.Med. 286:223, Feb. 1972.
5. Golde, D.W., Finley, T.N. and Cline, M.J. Production of colony stimulating factors by Human Macrophages. Lancet. Dec.30, 1972. pp 1397-1399.

1003539564

Theodore N. Finley, M.D.

13. Budget (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional.		
Dr. Theodore N. Finley	25	
Dr. W. Lawrence Drew	20	
Robert Hoo, Ph.D.	100	
Dr. David W. Golde		
Fringe benefits at 14% (R. Hoo Ph.D)		
Technical		
Research Tech II	100	
Research Tech I	50	
Secretary	50	
Fringe benefits at 14%		
	Sub-Total	
B. Consumable Supplies (list by categories)		
Tissue culture media	5,000	
Glassware	2,500	
Isotopes	1,500	
Tissue culture (tubed)	3,500	
Reagents (Antisera antigens etc)	1,000	
Electron Microscope supplies & rental	5,000	
	Sub-Total	\$18,500
C. Other Expenses (itemize)		
Travel	2,000	
Publications	1,000	
Volunteer subjects & medical expenses	1,000	
	Sub-Total	\$ 4,000
D. Permanent Equipment (itemize)		
Fiberoptic scope	4,000	
Freezer (-70°)	2,000	
Cytocentrifuge	1,000	
2 X CO ₂ incubators	5,000	
Tissue culture microscope	1,000	
Water baths, TC drum etc.	500	
	Sub-Total	\$13,500
E. Overhead (15% of A+B+C)		\$10,071
	TOTAL	\$90,712

Continued...

1003539565

Theodore N. Finley, M.D.

13. Budget (continued)

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	REDACTED	\$18,500	\$4,000	-0-	\$11,000	\$78,500
Year 3		\$18,500	\$4,000	-0-	\$11,500	\$81,000

It is understood that the applicant and institutional officers in applying for a grant had read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made".

Signature

Theodore N. Finley
Director of ProjectTelephone 415-567-6600 Ext. 645

Signature

J. B. G. Federal
Executive DirectorTelephone 415-567-6600 Ext. 201

Signature

S. Thompson
ControllerTelephone 415-567-6600 Ext. 205

1003539566

CURRICULUM VITAE

Theodore N. Finley, M.D.

Date of Birth:

REDACTED

Place of Birth:Education and
Training:

University of Washington, B.S. (Chemistry)
 Johns Hopkins Medical School,
 Internship: San Francisco County Hospital,
 Residency: Univ. of Calif. Medical Center
 San Francisco County Hospital

REDACTED

and

Scholarships or
Fellowships Held:

Research Fellow, 1957-1958, University of Buffalo,
 Department of Physiology;
 Research Fellow, American Trudeau Society, University of
 California, 1958-1961

Positions Held:

Clinical Instructor in Medicine, Cardiovascular Research
 Institute, Univ. of California Medical Center, 1958-1961;
 Assistant Professor of Anesthesiology, Physiology and
 Biophysics, and Director of Anesthesiology Research,
 University of Washington School of Medicine, 1961;
 Associate Professor, University of Washington School of
 Medicine, July 1964;
 Associate Professor of Medicine, University of New Mexico
 School of Medicine, September 1964 - June 1968
 Associate Chief of Medicine and Director of the Pulmonary
 Laboratory, Department of Medicine, Mount Zion Hospital
 and Medical Center, June 1, 1968;
 Associate Clinical Professor, Univ. California Medical
 Center, San Francisco, June 1958
 Medical Director Pulmonary Laboratory Mount Zion Hosp. 1968

Research
Appointments:

See above

Board Certification:

Qualified for Internal Medicine

Professional and
Scientific Member-
ships:

REDACTED

1003539567

Publications:

1. Finley, T.N., C. Lenfant, P. Haab, J. Pilper and H. Rahn. Venous admixture of pulmonary circulation of anesthetized dog. *J. Appl. Physiol.* 15:419, 1960.
2. McIlroy, M.B., J. Butler and T.N. Finley. Effects of chest compression on reflex ventilation drive and pulmonary function. *J. Appl. Physiol.* 17:701, 1962.
3. Finley, T.N. The relative efficiency of gas transport in the lung in relation to the ventilation perfusion ratio. WADD Technical Report 60-1, 1960.
4. Swenson, E.W., T.N. Finley and S.V. Guzman. Unilateral hypoventilation during temporary ipsilateral pulmonary artery occlusion in man. *J. Clin. Invest.* 40:828, 1961.
5. Severinghaus, J.W., E.W. Swenson, T.N. Finley, M.T. Lategola and J. Williams. Unilateral hypoventilation produced in dogs by occluding one pulmonary artery. *J. Appl. Physiol.* 16:53, 1961.
6. Finley, T.N. The determination of uneven pulmonary blood flow from the arterial oxygen tension during nitrogen washout. *J. Clin. Invest.* 40:1727, 1961.
7. Finley, T.N., E.W. Swenson, J.H. Comroe, Jr. The cause of arterial hypoxemia at rest in patients with "alveolo-capillary syndrome". *J. Clin. Invest.* 41:618, 1962.
8. Gardner, R.E., and T.N. Finley. The effect of cardiopulmonary bypass on the mechanical properties of the lung and surface tension of the lung extracts. International Surgical Congress, Dublin, Summer 1961. *Bulletin de la Societe Internationale de Chirurgie* 5:542, 1962.
9. Finley, T.N. Pulmonary surface activity and the problems of atelectasis, wetting, foaming and detergency in the lung. *Anesthesia and Analgesia* 42:35, 1963.
10. Zehner, Harry Jr., Richard E. Gardner, and T.N. Finley. Pulmonary function following reconstruction of a previously closed major pulmonary artery. *Am. Rev. Resp. Dis.* 87:694, 1962.
11. Finley, T.N., T.R. Hill, J.J. Bonica. Effect of varying intrapleural pressure on pulmonary shunt through atelectatic dog lung. *Amer. J. Physiol.* Oct. 1963.
12. Finley, T.N., W.H. Tooley, E.W. Swenson, R.E. Gardner, and J.A. Clements. Pulmonary surface tension in experimental atelectasis. *Am. Rev. Resp. Dis.* 89:372, 1964.
13. Swenson, E.W., D.A. Funk, S.V. Guzman and T.N. Finley. Vascular shunts in the lungs: Clinical evaluation by temporary unilateral pulmonary artery occlusion. *J. Thoracic and Cardiovascular Surgery* 48:671, 1964.
14. Morgan, Thomas E., T.N. Finley, G.L. Huber and Helen Fialkow. Alterations in pulmonary surface active lipids during exposure to increased oxygen tension. *J. Clin. Invest.* 44:1737, 1965.
15. Morgan, Thomas E., Finley, Theodore N., and Fialkow, Helen. Comparison of the composition and surface activity of "alveolar" and whole lung lipids in the dog. *Biochem. Biophys. Acta.* 106:403, 1965.
16. Huber, G.L., Edmunds, L.H., and Finley, T.N. Acute effects of saline lavage on pulmonary mechanics and morphology. *Surgical Forum* 17:113, 1965.
17. Finley, T.N., Swenson, E.W., Curran, W.S., Huber, G.L. and Ladman, A.J. Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. *Ann. Int. Med.* 66:651, 1967.

1003539568

Theodore N. Finley, M.D.
Publications - continued.

18. Ladman, A.J., Pratt, S.A., and Finley, T.N. Exfoliative cytology of the lung alveolus; Preliminary electron microscopic observations on cells obtained in vivo from human lungs. 25th Annual EMSA Meeting.
19. Finley, T.N., Ladman, A.J., Brewer, L., and McKay, M.E. A morphological and lipid analysis of the alveolar lining material in the dog. *J. Lipid Res.* 9:357, 1968.
20. Finley, T.N., Simson, G., McKay, M.B., and Lovekin, W.S. Effect of high hematocrit on ventilation at 5316 feet. 9th Annual Aspen Conference.
21. Finley, T.N. Anesthesia and Atelectasis. Editorial Views, *Anesthesiology*, 29:5, Sept-Oct. 1968.
22. Pratt, S.A., Finley, T.N., Ladman, A.J., Smith, M. A comparison of alveolar macrophages and pulmonary surfactant (?) obtained from the lungs of human smokers and nonsmokers by endobronchial lavage. *Anat. Rec.* 163:497, 1969.
23. Finley, T.N. Acute Pulmonary Embolism. *Current Therapy*, 1970.
24. Pratt, S.A., Smith, M.H., Ladman, A.J. and Finley, T.N. The ultrastructure of Alveolar Macrophages from Human Cigarette Smokers and Nonsmokers. *Lab. Invest.* 24:331, 1971.
25. Mann, P.E.G., Cohen, A.B., Finley, T.N., and Ladman, A.J. Alveolar Macrophages: Structural and Functional Differences between Nonsmokers and Smokers of Marijuana and Tobacco. *Lab. Invest.* 25:111, 1971.
26. Huber, G.L., Edmunds, L.H.Jr., and Finley, T.N. Effect of Experimental Saline Lavage on Pulmonary Mechanics and Morphology. *Am. Rev. Resp. Dis.* 104:337, 1971.
27. Finley, T.N. and Ladman, A.J. Low Yield of Pulmonary Surfactant in Cigarette Smokers. *New Eng. J. Med.* 286:223, 1972.
28. Golde, D.W., Finley, T.N. and Cline, M.J. Production of colony stimulating factors by Human Macrophages. *Lancet* (in press Jan. 1973).

1003539569

CURRICULUM VITAE

12

WILLIAM LAWRENCE DREW

BORN:

MARRIED:

REDACTED

CHILDREN:

EDUCATION:

Kegis High School, 33 East 4th St., New York, New York

Holy Cross College, Worcester, Mass.: Bachelor of Art.

Jefferson Medical College, Philadelphia, Pennsylvania
M.D.Experimental Pathology
Virology Ph.D.

TRAINING:

Straight Medical Internship - Jefferson Med. Coll.
Hospital 1962-63Assistant Residency in Medicine - Univ. Calif. Hosp.
San Francisco, Ca. 1966-68N.I.H. Fellow in Clin. Microbiology- Univ. Washington
Seattle, Wash. 1968-69N.I.H. Grant for part-time research in Endocrinology
During Senior Year in Medical School

BOARD CERTIFICATION:

American Board of Internal Medicine December 4, 1970

American Board of Medical Microbiology May 4, 1972

PRESENT:

Mount Zion Hospital, Director of Clin. Microbiology and
Virology Laboratory and Associate Chief, Medicine,
In charge of Infectious Diseases, and Hospital
Epidemiologist.Assistant Clinical Professor of Clinical Pathology and
Laboratory Medicine - Univ. of Calif. School of Medicine,
San Francisco, California 94122 July 1, 1971 - Present

MILITARY:

Commissioned as 1st Lieutenant U.S. Army Reserve Medical
Corps in May, 1963

Promoted to Captain, February, 1965

SOCIAL SECURITY

REDACTED

CALIFORNIA LICENSE

5/22/72

REDACTED

1003539570

- CURRICULUM VITAE -

13

MEMBERSHIP

REDACTED

AWARDS AND OFFICES

Scholarship - Regis High School
New York State College Scholarship
Francis Shain Scholarship - Jefferson Medical College

Alpha Omega Alpha - Jefferson Chapter
Hobart Hare Medical Honor Society - Jefferson Medical College
S. S. Cohen Prize in Medicine - 1962
Charles Labelle Prize as the Outstanding Graduate Student --- 1966

President, Student Council - Jefferson Medical College 1961-62
Chairman, Dean's Committee - Jefferson Medical College 1961-62

1003539571

- PUBLICATIONS -

1. Gilgore, S.C.; Drew, W. L. and Rupp, J.J.: Effects of salicylate on plasma free fatty acids and blood glucose. *Clinical Research* 10: 400 (4) December, 1972.
2. Gilgore, S.G.; Drew, W. L. and Rupp, J.J.: The effects of salicylate on plasma non-esterified fatty acids. *The American Jour. of the Med. Sciences* 245: 456 (4), April, 1963.
3. Gilgore, S. G.; Drew, W. L. and Rupp, J.J.: The effects of salicylate on aspects of carbohydrate and lipid metabolism. Presented at the Midwest Meeting of the American College of Physicians. Detroit, Michigan, November 21-23, 1963. Abstract published in *Annals of Internal Medicine*, 60: 333 (2) February, 1964.
4. Drew, W. L. : The effect of 5-fluoro-2'-deoxyuridine on Herpes Simplex virus growth and cytopathogenic effect. Ph.D. Thesis, 1966.
5. Drew, W. L. and Love, R.: Production of Herpes Simplex virus by HeLa cells treated with 5-fluoro-2'-deoxyuridine. *The American Jour. of Pathology*. 53: 169, July, 1968.
6. O'Toole, R.; Drew, W.L.; Dahlgren, B. and Beaty, H.: Outbreak of Methicillin-resistant Staphylococcus aureus infection in hospital and nursing homes. Presented at the Ninth Interscience Conference on Antimicrobial Agents and chemotherapy. October 27-29, 1969. *JAMA* 213: 257-263 (2), July 13, 1970.
7. Drew, W.L.; O'Toole, R. and Sherris, J.C.: Failure of Cloxacillin discs to detect resistance of Staphylococcus aureus to Penicillinase-resistant Penicillins. Presented at the Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy. October 27-29, 1969.
8. Drew, W. L. ; Barry, A.L.; O'Toole, R. and Sherris, J.C.: Reliability of the Kirby-Bauer Disc Diffusion Method for detecting "Methicillin-resistant" strains of Staphylococcus aureus. *Applied Microbiology* 24: 240-247. (2), August, 1972.
9. Drew, W.L.; Pedersen, A. N. and Roy, J.J.: An Automated slide staining machine. *Applied Microbiology* 23: 17-20 (1), January, 1972.

1003539572

CURRICULUM VITAE

Name: David W. Golde, M.D.

Address:

Date of Birth:

REDACTED

Marital Status:

EDUCATION:

REDACTED

B.S. Fairleigh Dickinson University, January,
M.D., CM McGill University, May,

LICENSURE & BOARD CERTIFICATION:

Diplomate, American Board of Internal Medicine, June, 1972
Diplomate, National Board of Medical Examiners, #86221
Maryland #C11431
Washington, D.C. #3776
California #15682

MILITARY SERVICE:

Surgeon, United States Public Health Service, July, 1967 -
July, 1970. Assigned to National Institutes of Health

HONORS AND SCHOLARSHIPS:

Alpha Omega Alpha Honor Society, 1965, McGill University
Phi Omega Epsilon Honor Society
University Scholar, McGill University, 1963-1966
New Jersey College of Surgeons Medical Scholarship, 1963-1965
Mosby Award, 1966
J. Francis Williams Prize in Medicine and Clinical Medicine,
McGill University, Department of Medicine, 1966

EXPERIENCE:

Assistant Research Chemist, General Foods Corporation
February - August, 1962

Research and Clinical Fellowship in Cardiology, San Juan de
Dios Hospital, San Jose, Costa Rica, Summers 1963, 1964, 1965

Consultant, Continuing Education and Training Branch, Division
of Regional Medical Programs, National Institutes of
Health, 1967-1968

1003539573

TRAINING:

Straight Internship in Medicine, University of California
Hospitals, San Francisco, California 1966-1967

Resident in Clinical Pathology, Clinical Center, National
Institutes of Health, Bethesda, Maryland 1968-1969

Hematology Fellow and Resident in Clinical Pathology,
Hematology Service, Clinical Center, National Institutes
of Health, Bethesda, Maryland 1969-1970

Resident in Medicine, University of California Hospitals,
San Francisco, California 1970-1971

Fellow, Cancer Research Institute and Resident in Medicine,
University of California Hospitals, San Francisco, California
1971-1972

FACULTY POSITIONS:

Instructor in Medicine, University of California School of
Medicine, July, 1972

Assistant Professor of Medicine, University of California School
of Medicine, July, 1973

PRESENTATIONS:

Systolic Phases of the Cardiac Cycle in Children, March, 1969
American College of Cardiology, New York City

The Albumin Agglutination Phenomenon, November, 1969
American Association of Blood Banks, Houston, Texas

Spectrum of Albumin Auto-Agglutinins, October, 1970
American Association of Blood Banks, San Francisco, California

Identification of a Morphologically Distinctive Cell in
Cultures of Neoplastic Plasma Cells, April, 1972.
American Federation for Clinical Research, Atlantic City,
New Jersey

Cultivation of Normal and Neoplastic Human Bone Marrow Leukocytes
in Liquid Suspension, June, 1972
Seventh Leukocyte Culture Conference, Quebec, Canada

Medical Oncology Training in the Cancer Center, Sept., 1972
West Coast Cancer Symposium, San Francisco, California

1003539574

PUBLICATIONS:

1. Golde, D.W., Aetiology of Regional Enteritis. *Lancet* 1144-1145, May, 1968.
2. Golde, D.W. and Epstein, W., Mixed Cryoglobulins and Glomerulonephritis. *Annals of Internal Medicine* 66:6, 1221-1227, Dec., 1968.
3. Golde, D.W., McGinniss, M.H., and Holland, P.V., Mechanism of the Albumin Agglutination Phenomenon. *Vox Sanguinis* 16: 465-469, June, 1969.
4. Zierdt, C. and Golde, D.W., Deoxyribonuclease-Positive *Staphylococcus epidermis* Strains. *Applied Microbiology* 20: 54-57, July, 1970.
5. Golde, D.W. and Burstin, L., Systolic Phases of the Cardiac Cycle in Children. *Circulation* 42:6, 1029-1036, Dec., 1970.
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7. Golde, D.W., McGinniss, M.H., and Holland, P.V., Serum Agglutinins to Commercially Prepared Albumin. *American Journal of Clinical Pathology* 55: 655-658, June, 1971.
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Ph.D. thesis: The effect of niacin and thiamine on carbohydrate
metabolism and nicotinamide coenzyme levels in
staphylococci.

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#916-GOLDSTEIN

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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

July 2, 1973

Grant Application No. 916

To: The committee comprising Drs. Loosli, Sommers and
Wyatt

Subject: Robert A. Goldstein, M.D., V. A. Hospital, Washington,
D. C.
New application No. 916
"Immunologic Functions of Human Alveolar Macrophages"

History

This proposal was Case No. 160, in the name of Bernard
W. Janicki, and formal application was encouraged.

Application No. 916 requests \$29,900 plus two additional
years.

Document Submitted

Attached is application dated 6/25/73 (41 pages).



F.W.N.

FWN:wg
Encl.

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Comm.

Dr. Loosli

Dr. Sommers

Dr. Wyatt

CHRONIC PULMONARY DISEASES

No. 916

JUN 25 1973

RESEARCH PROPOSAL

"IMMUNOLOGIC FUNCTIONS OF HUMAN ALVEOLAR MACROPHAGES"

Robert A. Goldstein, M.D.

Bernard W. Janicki, Ph.D.

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Dr. Loosli
Dr. Sommers
Dr. Wyatt

CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 50TH STREET
NEW YORK, N. Y. 10022

(212) 421-8885

Application for Research Grant

(Use extra pages as needed)

Date: 6/25/73

1. Principal Investigator (give title and degrees):

Robert A. Goldstein, M.D., Chest Physician, Veterans Administration Hospital, Washington, D.C.

Bernard W. Janicki, Ph.D., Chief, Pulmonary Immunology Research Laboratory, Veterans Administration Hospital, Washington, D.C.

2. Institution & address:

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3. Department(s) where research will be done or collaboration provided:

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Veterans Administration Hospital
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4. Short title of study:

Immunologic Functions of Human Alveolar Macrophages

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

The overall objective of this project is to gain insight into the role of the lung as an immunologic organ by critically examining selected biologic functions of the human alveolar macrophage. Furthermore, an attempt will be made to examine the influence of certain disease processes and environmental factors on these functions.

The specific aim of this project is to describe the immune reactivity of alveolar macrophages in patients with pulmonary infections (acute and chronic), in patients with obstructive lung disease (bronchitis and emphysema), and in patients with lung cancer. We also aim to define the qualitative and quantitative effects of cigarette smoking, the effects of extrapulmonary diseases (diabetes, cirrhosis, alcoholism, etc.), and the effects of therapy and environmental alterations on the functional capacity of alveolar macrophages.

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8. STATEMENT OF WORKING HYPOTHESIS

A. Background

In recent years, studies in experimental animals have lent considerable support to the concept that one of the most important nonrespiratory functions of the lung is its role in both the establishment and maintenance of immunity. The concept of local lung antituberculosis immunity developed by Dannenberg (1) has been supported recently by Barclay *et al.* (2) who have shown that rhesus monkeys immunized by means of aerosolized BCG achieved protection against a subsequent challenge with virulent tubercle bacilli without, at the same time, manifesting significant delayed skin hypersensitivity to PPD. Barbaras and Willoughby (3) have shown that rabbits immunized by aerosolized bovine serum albumin were able not only to manifest systemic humoral responses but also developed pathology in the lung similar to that experienced by persons with extrinsic allergic alveolitis.

Moreover, other investigators have shown that alveolar macrophages play an important role in antigenic processing by the lung and also constitute a functionally unique cell population when compared with other macrophages in the reticuloendothelial organs of the body. Leake *et al.* (4) showed that alveolar macrophages and peritoneal macrophages of rabbits were constitutively different in their enzymatic activities. Holub and Hauser (5) found that intrapulmonary instillation of sheep red blood cells into rabbits produced systemic humoral immunity by eliciting primarily an alveolar macrophage response with very little involvement of mediastinal lymph nodes. Ford and Kuhn (6,7) demonstrated that, following intranasal immunization with sheep red blood cells, rabbit alveolar macrophages were capable of synthesizing immunoglobulins and concluded that these cells were an important source of some of the immunoglobulins in respiratory secretions.

We conclude that the immunologic functions of the lung are more important than heretofore recognized and that the alveolar macrophages comprise one of the most important cell populations in mediating immune responses by the lung.

Attempts to extend such studies to humans have been limited by the unavailability of a viable alveolar cell population to study. Recently however, the widespread use of fiberoptic bronchoscopy in the diagnosis and treatment of pulmonary disorders has permitted for the first time examination of cell populations obtained from the most peripheral portions of the human lung. The technique of bronchopulmonary lavage, which had been previously utilized in the treatment of persons with alveolar proteinosis, status asthmaticus and cystic fibrosis (8,9), has been utilized for limited studies in healthy persons and has demonstrated the feasibility of this technique for the recovery of viable alveolar macrophages (10,11). To date, however, few studies of the immune function of these cells are available even though preliminary data suggest that techniques developed from the study of alveolar

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macrophage function in animals can be applied in humans (12).

B. Rationale

It has been suggested that cigarette smoking is not only of specific etiologic significance but also exerts a major deleterious influence in a variety of pulmonary diseases, including chronic bronchitis, emphysema and lung cancer (13). Because attempts to change the smoking habits of persons with these diseases have been largely unsuccessful, it seems imperative to develop specific information about the relationship between cigarette smoking and specific biologic functions of the lung in order to provide meaningful data relevant to reducing the purported health hazards of smoking.

The influence of smoking has been examined in animal and human experiments by a variety of morphologic, physiologic and biochemical parameters. Green and Carolin (14) showed that cigarette smoke depressed the phagocytic activity of alveolar macrophages in rabbits. Martin (15) showed that alveolar macrophages from otherwise healthy smokers contained an increased quantity of abnormally appearing cytoplasmic inclusions and of lysozomal enzymes. Yeager (16) demonstrated that smoke depressed protein synthesis by rabbit alveolar macrophages. On the other hand, Holt and Keast (17) showed that macrophages exposed to low levels of cigarette smoke for long periods had increased protein and RNA synthesis. Moreover, Harris and co-workers (11) found that in vitro phagocytosis and killing of Staphylococcus aureus by alveolar macrophages from smokers and non-smokers proceeded equally well, although smokers' cells had higher resting energy requirements. Little is known however, about the influence of cigarette smoking upon the immune functions of human alveolar macrophages. Warr and Martin (18), in a preliminary study, concluded that alveolar macrophages from otherwise healthy smokers had lost their ability to recognize and respond to specific antigens. Review of their data reveals that comparative experiments were not actually performed. The increased number of alveolar macrophages which are customarily obtained from smokers was not considered when migration chambers were prepared. Furthermore, in order to achieve statistical significance, a longer than usual period of migration (48-72 hours) was permitted. Collectively, these results suggest that further observations are necessary.

The present study is intended to resolve some of these questions. Sophisticated technology from animal experiments can be applied to the study of immune function of human alveolar macrophages. Differential effects related to underlying pulmonary or extrapulmonary diseases can be quantitated. Comparative knowledge based on quantitative and qualitative aspects of smoking can be achieved. The influence of environmental change (either acute or chronic) and therapy also can be examined.

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C. Hypothesis

Based on experimental animal studies and upon very preliminary studies in humans, (10-12, 19-22), it is our hypothesis that the alveolar macrophage plays an important role in the immunologic function of the lung. Appreciation of the lung as an immunologically competent organ has been generally under-emphasized. Although characterization of this function in humans has been impeded by lack of material to study, the widespread clinical use of fiberoptic bronchoscopy and the safety of local lung lavage, have made possible the examination of this function in living subjects. Implications about the adverse effects of cigarette smoking upon this function remain to be critically examined in humans. In addition to the influence of smoking, pulmonary and extrapulmonary disease and environmental alteration must be examined in order to provide meaningful data about pathogenic mechanisms of human disease and potentially specific information required for the counseling of any one individual on the influence of cigarette smoking on his health.

D. Significance

Definition of the number, kind and functional status of cells obtained from local lung lavage in certain disease states when related to qualitative and quantitative aspects of cigarette smoking is expected to provide information that is directly relevant to clinical problems. Despite the importance of the lung as an immunologic organ, human studies, to date, have failed to elucidate the specific influence of cigarette smoking upon this function. For example, it is uncertain that alveolar macrophages obtained from smokers are any more or less capable of responding in an immunologically competent fashion to aerosol immunization or to pulmonary infection. Furthermore, it is not known whether heretofore described deficits attributed to smoke in animal experiments have any biologic relevance in relation to the production of human diseases. It has been suggested, for example, that emphysema, which can be produced in experimental animals by enzymatic destruction of lung tissue (23), is also caused in humans by a similar mechanism. Because alveolar macrophages, especially those obtained from smokers, contain some of these enzymes in increased quantities (15) it has been presupposed that this is of pathogenetic significance.

It also is anticipated that this study will provide a general clarification of the role of the alveolar macrophage in the immunologic function of the lung in relation to pulmonary and extrapulmonary disease, specifically to the influence of cigarette smoking. Many of the patients studied will have other illnesses such as hypertension, cirrhosis, and alcoholism, among others. Thus, if cigarette smoking alters alveolar macrophage function in a very minor way, it will be possible to compare and contrast results obtained from persons with a variety of diseases

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in order to elucidate the alterations that such co-existing events may create.

We feel that the present proposal offers an opportunity to extend animal experiments to the more relevant clinical condition, to describe in a detailed fashion the immune function of alveolar macrophages in humans, and to relate derangements in function to specific host and environmental factors.

It is hoped that clear demonstration of specific host or environmental influences and potential alteration by medical therapy or environmental change, may lead to more constructive personal counseling. Persons are generally more receptive to medical recommendations that are of direct personal relevance than to blanket recommendations based on studies of other people or experimental studies in animals.

9. DETAILS OF EXPERIMENTAL DESIGN AND PROCEDURES

A. Study Subjects

1. Source

Subjects will be selected from among those persons in whom diagnostic and/or therapeutic fiberoptic bronchoscopy is clinically indicated. In addition to the standard hospital consent forms, an additional explanation will be made to those persons selected for study and informed consent will be obtained prior to limited lung lavage. A sample of the consent form and a letter from the Human Studies and Research Committees at this institution granting approval for this study are appended as attachments 1 and 2 respectively.

A complete history, physical and laboratory examination including routine blood work, skin test series, chest roentgenogram, electrocardiogram and pulmonary function studies (including arterial blood gas analysis) will be performed. A detailed respiratory history and environmental exposure will be recorded utilizing the standard questionnaire developed for this purpose by the British Medical Research Council (see attachment 3). Cigarette consumption, both qualitative (filter and non-filter) and quantitative (daily amount and years exposure) will be recorded.

2. Types of Disease States

At the outset, only persons with chronic lung disease (bronchitis and emphysema) pulmonary infections (pneumonia and lung abscess) and lung cancer will be examined. Patients will be studied initially at a time when fiberoptic bronchoscopy appears to be diagnostically or therapeutically indicated. Subsequently, specific and controlled environmental and therapeutic changes will be made under closely supervised conditions in a Respiratory Care Unit. Thus, cessation of smoking will be enforced

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both for therapeutic and study purposes in this manner. Repeat studies will be performed usually within 2 to 3 weeks. Practically speaking, this is an appropriate time interval to establish optimum pulmonary toilet, antibiotic therapy, and other forms of medical care. In persons with lung cancer, fiberoptic bronchoscopy is often the initial diagnostic procedure and leads to subsequent evaluation regarding operability. This "lag" period usually extends 1-2 weeks; it is anticipated that a repeat study in these persons can be performed just prior to the time of surgery. In this manner, each person is expected to serve as his own control. Serial studies in the same person serve to keep constant as many human variables as possible. In contrast, single studies in a large group of persons are less satisfactory because of extreme biologic variations. No studies are anticipated at this time in healthy persons. There are two reasons for this, neither related to the potential scientific merits of such examinations. In the first place, the Human Studies Committee at this institution suggested (not insisted), that caution at the initiation of such a study appeared to be in the best interests of the Hospital and the patient. Since potential information of therapeutic value might be achieved in persons who would otherwise be bronchoscoped, the initial procedure could be of direct benefit to the patient and not be construed as a procedure performed for "research purposes" only. In the second place, the Committee felt that a decision should be made concerning the study of healthy persons in whom there would be no clinical indication for the procedure after sufficient information was obtained and evaluated in a patient population.

3. Bronchoscopy and Lung Lavage

Following an overnight fast, the subject is seated in a dental chair. Local anesthesia will be accomplished by application of viscous and liquid 1% xylocaine to the nasopharynx and vocal cords. The distal end of the fiberoptic bronchoscope will be passed under direct vision into the trachea. Following the indicated diagnostic evaluation and using an amount of local anesthetic not to exceed 250 to 300 mg xylocaine (as a 1% solution to a volume usually of 30 ml), the bronchoscope will be passed into the right or left lower lobe. A segmental local lavage, restricted to one area, then will be performed using 50 ml aliquots of sterile, pyrogen-free 0.9% saline which has previously been warmed to 37°C (24). Fluid will be allowed to flow in by gravity and removed by gentle suction with a syringe. It is anticipated that approximately one-half of each aliquot will be recovered and that no more than 6 aliquots will be utilized. Until the cell yield can be determined per aliquot in various disease states, we will initiate the series of experiments with only one 50 ml local lavage. Presumably following an initial series of studies, the actual number of aliquots necessary to achieve the desired cell numbers can be determined. In either instance we shall strive to use the least possible number and in no case exceed a

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total of 6 (or 300 ml). Following the procedure, patients will be observed in the usual manner; that is, no oral intake until the gag reflex has returned, and follow-up physical, laboratory and x-ray examinations will be performed as indicated. It is anticipated that the lavage will add approximately 15 minutes to the overall bronchoscopic examination.

As recommended by the manufacturer, the flexible tube of the bronchoscope will be sterilized by immersion in 30% ethanol overnight. After soaking, it will be wiped with a gauze sponge soaked in alcohol and subsequently dried with a sterile sponge and stored in a sterile cloth bag. The tube will be handled using sterile gloves while the lens cleanser and xylocaine jelly are applied prior to insertion. After each use, the flexible tube will be wiped with a gauze sponge soaked in 30% alcohol and the aspiration channel will be rinsed and brushed. These precautions are considered reasonable and are in keeping with attempts not to destroy the delicate optical fibers by more rigorous sterilization processes such as autoclaving and/or gas sterilization, which have been found to be harmful to the scope. Periodic cultures will be made to determine the effectiveness of the sterilization procedure.

4. Immunologic Profile

a. In Vivo Tests

Intradermal reactivity to a battery of test antigens including tuberculin PPD, streptokinase, Candida albicans, and mumps, will be determined in all study subjects. The skin reactions will be measured and recorded 48 hours after injection.

b. In Vitro Tests

To determine in vitro cellular immune responses, a heparinized venous blood sample will be drawn from each study subject. Leukocyte-rich plasma (LRP) will be obtained after erythrocyte sedimentation on standing at room temperature for 1 to 2 hours. The LRP will be processed for study as described below and the sedimented blood will be centrifuged at 2,100 g for 20 minutes at 5 C to obtain platelet-depleted, autologous plasma.

1) Lymphocyte Blastogenesis

The cells of the LRP will be sedimented by centrifugation and resuspended to contain approximately 1×10^6 lymphocytes in 3 ml of culture medium containing 80% medium 199 and 20% autologous, cell-free plasma, as described previously (25). The response of the cultured cells to selected specific antigens and to non-specific mitogenic stimuli, such as phytohemagglutinin, concanavalin A, and pokeweed mitogen, will be examined. Triplicate 3 ml cultures will be prepared for each

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stimulus; cultures to which no stimulus was added will be used as negative controls. After an appropriate period of incubation at 37 C, each culture will be subjected to a 3 hour pulse with 0.03 μ Ci of 3 H thymidine. The cultures then will be processed (26) for assay of radioactive trichloroacetic-acid precipitable material. Lymphocyte blastogenesis will be expressed as an S/C ratio which is calculated by dividing the radioactivity (DPM) of the cultures to which the stimulus was added (S) by that of the unstimulated negative control (C) cultures.

2) Lymphokine Production

The release of migration inhibitory factor (MIF) into the medium on culture of blood lymphocytes with selected, specific, antigens will be used as an indicator of lymphokine production. The methods described by Rocklin and his coworkers (27) will be used for this purpose. Blood lymphocytes in the LRP will be purified by passage through a nylon-cotton column and subsequently cultured for 4 days at 37 C in medium with and without specific antigens. The culture medium will be changed on a daily basis; the cell-free supernatant culture fluids, collected daily, will be appropriately pooled, dialyzed, and concentrated by freeze-drying. MIF activity will be assayed routinely using oil-induced peritoneal exudate cells from normal guinea pigs by methods developed previously in this laboratory (28,29). Occasionally, as described below, a preparation with confirmed MIF activity will be used to examine the functional status of human alveolar macrophages.

B. Profile Studies of Alveolar Cells

The analysis of alveolar macrophage immunologic reactivity necessitates an assessment of their functional state. For this reason, the alveolar cells will be examined by various parameters, as described below, to develop a functional profile.

1. Cell Isolation Techniques

The pooled alveolar washings will be processed aseptically for study on the day of collection. For morphologic characterization of the cell suspension, the cells contained in a 5 ml aliquot of the pooled washings will be isolated by membrane filtration (5 μ porosity filter). The cells on the filter will be washed once with an equal volume of saline and once with 15 ml of 95% ethanol after which the wet filter pad will be transferred to a staining dish for subsequent processing. The remainder of the pooled washings will be centrifuged at 300g for 10 minutes at 4 C to sediment the cells. The packed cells will be washed twice by centrifugation using Hanks balanced salt solution (BSS) and

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finally resuspended in a minimal volume of BSS to provide an approximate 100-fold concentration. The cell populations in the final suspension will be enumerated by total and differential counts; macrophage viability will be determined by the eosin Y-exclusion test described by Hanks and Wallace (30).

2. Morphologic and Histochemical Characterization

a. Morphology

The alveolar cell preparation will be processed for morphologic examination by light microscopy. For this purpose, the cells isolated on the membrane filter will be fixed with 95% ethanol and stained with hematoxylin and eosin. The stained preparation will be dehydrated progressively with ethanol, clarified with xylene, and mounted on glass slides. As described by Martin (15), the stained cells will be characterized on the basis of cytoplasmic inclusions, number of nuclei per cell, and cell diameter.

b. Histochemistry

The enzymatic capabilities of alveolar macrophages will be characterized qualitatively by histochemical techniques. As suggested by Dannenberg (1), an estimate of macrophage "activation" can be obtained from histochemical analyses of representative lysozomal and mitochondrial enzymes. For this purpose, the methods developed by Dannenberg and his associates (31) for study of rabbit alveolar macrophages will be modified and adapted. The concentrated alveolar cell suspension will be used to prepare smears on Mylar film or glass slides which will be air dried and stored in the cold for subsequent study. The β -galactosidase and cytochrome oxidase reactivity of the alveolar macrophage will be examined as representative lysozomal and mitochondrial enzymes, respectively, using the fixatives and substrates described by Dannenberg and his associates (31,32). As indicated for individual patients in the study, other macrophage enzymes will be examined using similar methodology. Also, as indicated, selected enzymes can be assayed quantitatively in lysates of alveolar macrophages using the methods described by others for human (15) and animal (4, 31-33) cells. It is planned that our capability in these techniques will be developed in collaboration with Arthur M. Dannenberg, Jr., M.D. of the Johns Hopkins University School of Medicine; confirmation of this arrangement is provided in the attached letter (Attachment 4).

3. Functional Characterization

a. Phagocytic Activity

The methods described by Massaro and his

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coworkers (34) to measure phagocytosis of polystyrene latex beads by rabbit alveolar macrophages will be modified and adapted for the present study. It is planned that approximately 15×10^6 macrophages from the concentrated alveolar cell suspension will be incubated with the beads at 37 C for 30 minutes. A 10% suspension of beads will be prepared as they described. Phagocytosis of the beads will be measured by counting the number of macrophages containing beads, and the number of beads/cell, in randomly-selected fields at a 400X magnification. Approximately 200 cells will be examined from each preparation; the results will be expressed as a percentage of phagocytizing macrophages and as a percentage of cells containing 10 or more beads/cell, as suggested previously (34). As required for a more precise measure of phagocytosis of the beads, the quantitative assay technique described by Weisman and Korn (35) will be employed. For this determination, the alveolar macrophages will be sedimented by centrifugation at the end of the incubation period, washed to remove extracellular beads and extracted with dioxane to solubilize the phagocytized beads. The absorbance of the dioxane extract at 259 m μ will be used to provide a quantitative measure of latex contained in the macrophage preparations.

b. Metabolic Activity

Oxygen consumption will be measured manometrically in a Gilson differential respirometer as a representative parameter of the alveolar macrophages' metabolic activity. An aliquot of the concentrated alveolar cell suspension will be incubated in Krebs-Ringer-phosphate solution which contains glucose as the metabolic substrate. The procedures for preparation of solutions, operation of the instrument, and calculations of data will be the same as those described by Umbreit and his collaborators (36); oxygen consumption will be expressed as μ l O₂/hr/10⁶ cells.

4. Immunologic Reactivity

a. Direct Migration Test

Specific antigen - induced inhibition of macrophage migration, and accepted in vitro correlate of delayed-type hypersensitivity (37), will be employed as a measure of alveolar macrophage immunologic reactivity. The test antigens will be the same as those used in the in vivo tests to develop the study subject's immunologic profile, as described above. The methods used in this laboratory (28) for study of guinea pig macrophage migration inhibition will be modified and adapted for the present study. The concentrated alveolar cell suspension will be supplemented, as required, with leukocyte-rich plasma isolated from the subject's peripheral blood as described above to provide a 5 to 10% lymphocyte concentration. Non-heparinized

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capillary tubes will be filled with the suspension, sealed at one end with paraffin wax, and centrifuged. The tubes then will be cut just below the cell-fluid boundary and the sections containing the packed cells will be placed in sterile silicone grease on the bottom of a tissue culture dish. Each dish will contain 4 to 6 capillary tubes and will receive 4 ml of culture fluid. In initial experiments, the culture fluid will contain Eagle's minimal essential medium, 15% autologous serum, 1% L-glutamine, penicillin G (100 units/ml) and streptomycin (0.1 mg/ml); the composition of the culture fluid may be modified, as required, for later experiments to provide an optimal environment for the macrophages. The dishes will be sealed with transparent tape and incubated at 37 C for 24 hours after which migration of cells from the capillary tip will be recorded by tracing a 15.6X projection of the area surrounding the capillary tip. The area of cell migration will be measured by planimetry of the tracing. Macrophage migration in the presence of the test antigens will be expressed as a percentage of the migration area of the same cell suspensions in medium without the test antigens. At least 4 capillaries each for control and test antigen cultures will be measured for each suspension.

b. Response to Lymphokine

An indirect migration inhibition test also will be used to examine the immunologic reactivity of alveolar macrophages. In this test, the response of alveolar macrophages to migration inhibitory factor (MIF) will be evaluated. The MIF lymphokine will be produced by incubating column-purified peripheral blood lymphocytes from a tuberculin hypersensitive donor with PPD using the methods described above. Capillary tubes containing the study subject's alveolar macrophages will be cultured both in a control and MIF-containing medium. Measurement of migration areas and calculation of migration inhibition by MIF will be as described above for the direct migration test.

5. Statistical Analysis

At the outset, no healthy subjects will be studied to provide "control data". It is anticipated, however, that internal group comparisons of persons with a variety of diseases can be made at a later date. Thus, similar parameters can be compared in subjects with the same diseases at different times by a paired t test (38).

6. Timing of Studies

It is anticipated that the early phases of this project will be devoted to the modification and adaptation of

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existing methodology from animal models for study of human alveolar macrophages. This will necessitate preliminary studies in humans to obtain baseline information concerning cell yield and other technical aspects of the experiments. As indicated earlier, the assistance to be provided by Dr. Dannenberg should facilitate development of our capability for histochemical methodology. Dr. Donald J. Massaro, Chief of the Pulmonary Disease Section at this hospital, will provide similar assistance in adapting methods for study of the phagocytic and metabolic activities of human alveolar macrophages. Our prior experience with methods for study of cell-mediated immune phenomena in related human and animal models will be employed to finalize the tissue culture methods for migration studies of human alveolar macrophages. This early phase should require no more than 3-4 months, following which we shall be able to perform complete functional profiles in selected patients. Subsequent to that time it is planned that 2 complete studies will be attempted each week, in anticipation that at least one complete set of results will be obtained weekly. On this basis, we project that approximately 64 complete studies will be performed during the first year. It is anticipated that once an adequate number of experiments has been performed in patients with the aforementioned disease states, the protocol will be expanded for the study of healthy volunteer subjects. The latter group should provide a more complete evaluation of alveolar macrophage function. Since the study of healthy volunteers will require direct financial compensation for each subject, we anticipate an additional request in subsequent budgets for this purpose. We expect to complete the entire series of experiments within 3 years.

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C. Literature Cited

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1003539597

Washington Veterans Administration Hospital (WVAH)
George Washington University Medical Center (GWUMC)

Consent for Research

1. I, _____, hereby agree to participate in a program of studies under the supervision of Dr. Robert Goldstein. I understand that while the program will be under the supervision of Dr. Robert Goldstein, other professional persons who work with him may be designated to assist him or to act for him.
 2. The studies have been described and their purpose explained to me by Dr. Robert Goldstein. I understand that the studies will involve or require some or all of the following tests or procedures: in addition to having a tube passed through my nose (bronchoscope) and into my lungs, from 50 to 300 ml (as little as 2 ounces or as much as 10 ounces) of fluid will be allowed to go into my lung (segmental lung lavage) and subsequently be removed.
 3. I understand that the study includes the following risks: that some of the fluid may remain in my lungs for as much as one or two days following the procedure, and that it is possible I may experience some increased difficulty breathing following the procedure.
- I voluntarily accept the risks of these studies. I further understand that every precaution has been and will be taken to remove and reduce risk.
4. I authorize WVAH/GWUMC to keep, preserve, use or dispose of the results of the studies in which I have agreed to participate.
 5. I understand that my participation in the study may be terminated upon my wishes or at the discretion of Dr. Robert Goldstein.

Date_____
Signature

I, the undersigned, have defined and fully explained the studies involved to the above.

Date_____
Investigator's Signature

I, the undersigned, having no special interest in the research proposal, and having heard the explanation given by Dr. _____ to _____, certify that the explanation seemed complete and was apparently understood by the patient/subject and that he/she signed this form in my presence.

Date_____
Witness Signature

1003539598

REPORT OF SUBCOMMITTEE ON HUMAN STUDIES

TITLE DESCRIPTIVE OF PROPOSAL, APPLICATION, PROCEDURE, DRUG USAGE, STUDY, ETC.

"Immunologic functions of human alveolar cells"

NAME OF PRINCIPAL INVESTIGATOR

Robert A. Goldstein, M.D.

NAME AND LOCATION OF VA INSTALLATION

VAH, Washington, D.C.

1. This is to certify that the grant application entitled "Immunologic functions of human alveolar cells"
(Proposal, application, procedure, drug usage, study)

identified above has been reviewed by the Research and Education Subcommittee on Human Studies appointed to consider clinical research proposals and other investigations involving human beings.

2. The Subcommittee on Human Studies has considered specifically:

- (a) the rights and welfare of the individual(s) involved,
- (b) the appropriateness of the methods to be used to secure informed consent, and
- (c) the risks and potential medical benefits of the investigation.

3. The collective judgment of the Subcommittee is that: (Check (a) or (b))

- ☒ (a) the proposed drug usages and/or procedures are considered appropriate, and approval is recommended.
- ☐ (b) the proposed drug usages and/or procedures are considered inappropriate, as presented, and approval is not recommended for the reasons stated in the attached memorandum.

4. This proposal is consistent with the policy set forth in Chapter 8, DM&S Manual M-2, Part XIV.

1003539599

DATE OF SUBCOMMITTEE ACTION

4/9/73

SIGNATURE of the Subcommittee on Human Studies

WILLIAM MCFARLAND, M.D.

FINAL ACTION

☒ APPROVED

DATE SIGNED

5/3/73

SIGNATURE OF CHAIRMAN OF RESEARCH AND EDUCATION COMMITTEE

B.W. JANICKI, Ph.D.

☐ DISAPPROVEDVA FORM
APR 1967

10-1223

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ATTACHMENT #3

APPENDIX A

Following is the questionnaire used in the study, essentially the one approved in 1966 by the British Medical Research Council's Committee on Research into Chronic Bronchitis (modifications consisted of substitutions of U.S. for English idiom, e.g., "mucus" for "phlegm", and the addition of two preliminary data, age and sitting height).

QUESTIONNAIRE ON RESPIRATORY SYMPTOMS

SURVEY OR HOSPITAL NUMBER..... Date of interview _____
 Date of birth _____
 NAME..... Age _____
 (Surname) Sex _____
 Civil state _____
 (First names) Standing height (inches) _____
 Sitting height (inches) _____
 ADDRESS..... Weight (lbs.) _____
 Occupation _____
 Race _____
 Name of interviewer _____

Use the actual wording of each question. Put X in appropriate square after each question. When in doubt record 'No'.

PREAMBLE I am going to ask you some questions, mainly about your chest. I would like you to answer 'YES' or 'NO' whenever possible.

COUGH

1. Do you usually cough first thing in the morning (on getting up*) in bad weather?

☐ ☐
 Yes No

Count a cough with first smoke or on first going out of doors.
Exclude clearing throat or a single cough.

* For subjects who work by night.

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Page 2

3. Do you usually cough during the day--or at night--in bad weather?

☐ ☐
Yes No

Ignore an occasional cough.

If 'No' to Both questions 1 and 3, go to question 6.

If 'Yes' to either question 1 or 3:

5. Do you cough like this on most days (or nights*) for as much as three months each year?

☐ ☐ ☐
Yes No N.A.

MUCUS

6. Do you usually bring up any mucus from your chest first thing in the morning (on getting up*) in bad weather?

☐ ☐
Yes No

Count mucus with the first smoke or on first going out of doors.
Exclude mucus from the nose. Count swallowed mucus.

8. Do you usually bring up any mucus from your chest during the day--or at night--in bad weather?

☐ ☐
Yes No

Accept twice or more.

If 'No' to both questions 6 and 8, go to question 12a.

If 'Yes' to either question 6 or 8:

10. Do you bring up mucus like this on most days (or nights*) for as much as three months each year?

☐ ☐ ☐
Yes No N.A.

- 12a. In the past three years have you had a period of (increased**) cough and mucus lasting for three weeks or more?

No ☐

If 'No' to question 12a, go to question 13.

If 'Yes' to question 12a:

Yes--1 period ☐

- 12b/c. Have you had more than one such period?

Yes--2 or more periods ☐

* For subjects who work by night.

** For subjects who usually have mucus.

13. Have you ever coughed up blood?

No ☐

If 'No' to question 13, go to question 14a.

If 'Yes' to question 13:

Yes--in past year ☐

- 13a. Was this in the past year?

Yes--not in past year ☐

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Page 3

BREATHLESSNESS

- 14a. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? Disabled* ☐

If 'No' to question 14a, go to question 15a. No--a ☐

If 'Yes' to question 14a:

- 14b. Do you get short of breath walking with other people of your own age on level ground? No--b ☐

If 'No' to question 14b, go to question 15a.

If 'Yes' to question 14b:

- 14c. Do you have to stop for breath when walking at your own pace on level ground? No--c ☐

* Disabled from walking by any conditions other than heart or lung disease. Yes--c ☐

WHEEZING

- 15a. Does your chest ever sound wheezing or whistling? No ☐

If 'No' to question 15a, go to question 16a.

If 'Yes' to question 15a: Yes, but not most days (or nights) ☐

- 15b. Do you get this most days--or nights? Yes, most days (or nights) ☐

- 16a. Have you ever had attacks of shortness of breath with wheezing? No attacks ☐

If 'No' to question 16a, go to question 17.

If 'Yes' to question 16a: No ☐

- 16b. Is/was your breathing absolutely normal between attacks? Yes ☐

WEATHER

17. Does the weather affect your chest?

Only record 'Yes' if adverse weather definitely and regularly causes chest symptoms. No ☐

If 'No' to question 17, go to question 18.

If 'Yes' to question 17: Yes ☐

- 17a. Does the weather make you short of breath? No ☐

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17b. Specify type of weather, e.g. fog, damp, cold, heat, other ☐

NASAL CATARRH

18. Do you usually have a stuffy nose or mucus at the back of your nose in the winter? ☐ ☐

19. Do you have this in the summer? ☐ ☐

If 'No' to both questions 18 and 19, go to question 21.

If 'Yes' to either question 18 or 19:

20. Do you have this on most days for as much as three months each year? ☐ ☐ ☐

CHEST ILLNESSES

21. During the past three years have you had any chest illness which has kept you from your usual activities for as much as a week? ☐ ☐ ☐

If 'No' to question 21, go to question 22.

If 'Yes' to question 21:

21a. Did you bring up more mucus than usual in any of these illnesses? No ☐

If 'No' to question 21a, go to question 22.

If 'Yes' to question 21a:

1 illness ☐

21b. How many illnesses like this have you had in the past three years?

2 or more illnesses ☐

HAVE YOU EVER HAD:

22. An injury or operation affecting your chest? ☐

23. Heart trouble? ☐

24. Bronchitis? ☐

25. Pneumonia ☐

26. Pleurisy? ☐

27. Pulmonary tuberculosis? ☐

28. Bronchial asthma? ☐

29. Emphysema? ☐

30. Bronchiectasis? ☐

31. Other chest trouble? ☐

* Code: 0=no; 1=once; 2=twice;nine or more times.

** Code 0=no; 1=yes

Give relevant details after each positive answer.

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TOBACCO SMOKING

35a. Do you smoke? ☐ ☐
 Record "Yes" if ☐ Yes ☐ No
regular smoker (as defined in question 35b) up to one month ago.

If 'No' to question 35a, ask question 35b.

If 'Yes' to question 35a:

Do you inhale the smoke?

☐ ☐
 Yes No

Would you say you inhale the smoke slightly (S), moderately (M), deeply (D)?

☐ ☐ ☐
 S M D

How old were you when you started smoking regularly?

_____ years old

How many manufactured cigarettes do you usually smoke per day?

_____ per working day

_____ at weekends

How much tobacco (oz/g) do you usually smoke per week in hand-rolled cigarettes?

How much pipe tobacco (oz/g) per week were you smoking before you gave up?

How many cigars do you usually smoke per week?

Specify large (L) or small (S).

35b. Have you ever smoked as much as one cigarette a day (or one ounce of tobacco a month) for as long as a year?

☐ ☐
 Yes No

CODING FOR SMOKING HISTORY

Before coding refer to instructions

Smoking history

Never smoked ☐

Ex-smoker ☐

Present smoker--does not inhale ☐

Present smoker--inhales slightly ☐

Present smoker--inhales moderately ☐

Present smoker--inhales deeply ☐

Type of smoker

Cigarettes only ☐

Pipe only ☐

Cigars only ☐

Cigarettes and pipe/cigars ☐

Cigars and pipe ☐

Non-smoker ☐

Amount smoked per day* (average including weekends)

Cigarette tobacco:

Nil ☐

1-4 g ☐

5-14 g ☐

15-24 g ☐

25 g or more ☐

* 1 oz of pipe tobacco = 28 cigarettes
= 28 g

1 small cigar = 2 cigarettes

1 large cigar = 5 cigarettes

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If 'No' to
question 35b, go
to question 38.

If 'Yes' to question
35b:

How old were you when you
started smoking regularly?

_____ years old

How old were you when you last
gave up smoking?

_____ years old

How many manufactured cig-
arettes per day were you smoking
before you gave up?

_____ per working day

_____ at weekends

How much tobacco (oz/g) per
week were you smoking in hand-
rolled cigarettes before you
gave up?

How much pipe tobacco (oz/g) per
week were you smoking before you
gave up?

How many cigars per week were
you smoking before you gave up?

Specify large (L) or small (S).

OCCUPATION

Record on dotted lines number of years
in which subject has worked in any of
these industries.

38. Have you ever worked in a
dusty job?

39. At a coalmine ☐ ☐
Yes No

Pipe/cigar tobacco:

Nil

1-4 g

5-14 g

15-24 g

25 g or more

Age started
(years)

Code XX
if a non-
smoker

Age stopped
(years)

Code YY
if a present
smoker

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40. In any other mine..... ☐ ☐
Yes No
41. In a quarry..... ☐ ☐
Yes No
42. In a foundry..... ☐ ☐
Yes No
43. In a pottery..... ☐ ☐
Yes No
44. In a cotton, flax or
hemp mill..... ☐ ☐
Yes No
45. With asbestos..... ☐ ☐
Yes No
46. In any other dusty job. ☐ ☐
Yes No
- If 'Yes', specify
.....
Total number of years in
dusty job?..... ☐ ☐

47a. Have you been exposed regularly
to irritating gas
or chemical fumes?

Yes, 47a and b ☐No, 47a and b ☐

If 'Yes', give details
of nature and duration.

..... Yes

47a } ☐

..... No

47b

47b. Have you ever been off work
for a shift or
longer following
acute exposure to
gases or fumes?

No

47a } ☐

Yes

47b

If 'Yes', give details of
nature and duration.

.....

.....

.....

.....

TIME _____ AMBIENT TEMPERATURE °C _____

FEV

3 _____ 4 _____

5 _____

☐ ☐ ☐

VITAL CAPACITY

3 _____ 4 _____

5 _____

☐ ☐ ☐

FEV %VC

☐ ☐

MMEF

3 _____ 4 _____

5 _____

☐ ☐ ☐

1003539606

ATTACHMENT #4

THE JOHNS HOPKINS UNIVERSITY

DEPARTMENT OF RADIOLOGICAL SCIENCE

SCHOOL OF HYGIENE AND PUBLIC HEALTH

615 North Wolfe Street • Baltimore, Maryland 21205

June 6, 1973

Dr. Bernard Janicki
Veterans Administration Hospital
50 Irving Street, N.W.
Washington, D.C. 20422

Dear Bernard:

We shall be happy to train your technician in our laboratory in the various histochemical procedures we use, and be of any other assistance that may be required to launch your proposed program on the alveolar macrophages of man.

With very best regards,

Sincerely,

Art

Arthur M. Dannenberg, Jr., M.D.
Associate Professor of
Radiobiology and Pathology

AMD:ms

Enclosures: Reprints

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10 Space and facilities available (when elsewhere than item 2 indicates, state location):

All phases of this project will be conducted at the Washington, D.C., Veterans Administration Hospital. The selection of study subjects, as well as the bronchoscopic and lavage procedures, will be under the direct supervision of Robert A. Goldstein, M.D. He has performed and/or supervised fiberoptic bronchoscopy during the course of routine diagnostic and therapeutic evaluations at the VA Hospital since July 1, 1972. A sufficiently varied clinical population is available for study; during the period July 1, 1972 through February 28, 1973, 182 bronchoscopic procedures have been performed. These included 89 patients with lung cancer, 26 with lung abscesses, 13 chronic bronchitics, 23 pneumonia patients, and 31 others with various pulmonary disorders. The Pulmonary section at this hospital is comprised of 35 in-patient beds including 10 for the explicit purpose of acute and chronic convalescent respiratory care. For the purposes of this study all clinical work will be performed in conjunction with a pulmonary fellow and Dr. Goldstein. Patients will also be available from two affiliated institutions, George Washington University Hospital and the Washington Hospital Center.

The laboratory studies will be performed in the Pulmonary Immunology Research Laboratory of the Washington DC VAH under the direct supervision of Bernard W. Janicki, Ph.D. This unit is fully equipped for the studies planned in this proposal; the necessary radioisotope counting equipment and animal facilities are available in shared and common facilities.

11: Additional facilities required.

NONE

12. Biographical sketches of investigator(s) and other professional personnel (append):

- A. Robert A. Goldstein, M.D.
- B. Bernard W. Janicki, Ph.D.

13. Publications. (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See Sections 12 A and B.

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SECTION 12A

CURRICULUM VITAE

NAME: Robert Arnold Goldstein

BORN:

RESIDENCE:

REDACTED

MARITAL STATUS:

EDUCATION:

Chester High School, Chester, Pa., R

DEGREES:

A. B. Brandeis University, Waltham, Mass., REDACTED

M. D. Jefferson Medical College, Phila. Pa. REDACTED

PROFESSIONAL
EXPERIENCE:Philadelphia General Hospital, Phila. Pa
Internship, Mixed Medical, 1966-67Veterans Administration Hospital, Wash. D. C.
Resident, Internal Medicine, 1967-69
Fellow, Pulmonary Disease, 1969-70Tripler Army Medical Center, Honolulu, Hawaii
Ass't Chief, Pulmonary Disease Service, 1970-71
Chief, Pulmonary Disease Service, 1971-72Veterans Administration Hospital, Wash, D. C.
Staff Physician, Pulmonary Disease Section and
Associate Chief, Pulmonary Immunology Research
Lab., 1972-ACADEMIC
APPOINTMENTS:University of Hawaii, School of Medicine
Honolulu, Hawaii, Ass't Clinical Professor of
Medicine, 1970-72George Washington University School of Medicine
Washington, D. C, Ass't Professor of Medicine
and Staff Physician, 1972-

CONSULTANT:

Queen's Hospital, Honolulu, Hawaii, 1970-72

National Institutes of Health, Pulmonary Medicine
Branch, Bethesda, Md. 1972-

Washington Hospital Center, Wash, D. C. 1973-

CERTIFICATION:

National Board of Medical Examiners, 1967

American Board of Internal Medicine, 1972

LICENSE:

Maryland, District of Columbia, Pennsylvania

MILITARY:

Major, United States Army, 1970-72

1003539609

MEMBER:

REDACTED

COMMITTEES:

Program Chairman, Hawaii Thoracic Society, 1971-72

Program Committee, Scientific Assembly on
Microbiology and Immunology, American Thoracic
Society, 1973-Medical Advisory Committee, American Lung Assoc.
Southern Maryland, 1973-

RESEARCH INTERESTS:

Pulmonary Disease and Immunology

PUBLICATIONS:

A. Journal Articles

1. Israel HL, Goldstein, RA: Metyrapone test in sarcoidosis. Amer Rev Resp Dis 98: 713-716, 1968.2. Israel, HL, Goldstein, RA: Advances in sarcoidosis. Derm Digest 7:77-85, 19683. Goldstein, RA, Israel, HL: An assessment of serum protein electrophoresis
in sarcoidosis. Amer J Med Sci 256: 302-313, 1968.4. Goldstein, RA, Israel, HL, Rawnsley, HM: Effects of race and stage of disease
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mittent elevations and seasonal effect. New Eng J Med 287: 649-650, 19729. Israel, HL, Goldstein, RA: Hepatic granulomatosis and sarcoidosis
Annals Int Med (In press)10. Goldstein, RA, Becker, KL, Israel, HL, Moore, CF: Urate metabolism in sar-
coidosis. Arch Int Med (In press)

B. Abstracts

1. Janicki, BW, Goldstein, RA, Aron, SA: Immunoelectrophoretic studies of the
precipitating antibody response in tuberculosis. Amer Rev Resp Dis 103: 894, 19712. Goldstein, RA, Yokoyama, M: Immunologic reactions in patients recovered from
malaria. Fed Proc 31: 931, 19723. Goldstein, RA, Janicki, BW, Schultz, KE: Reactivity of lymphocytes from
sarcoidosis patients to Kveim antigen in vitro. Clin Res 20: 378, 1972.4. Goldstein, RA, Israel, HL, Janicki, BW, Yokoyama, M: Serum immunoglobulin
D levels in sarcoidosis. Proceedings of the Sixth International Sarcoidosis
Conference, Tokyo, 1972 (In press)5. Goldstein, RA, Becker, KL, Israel, HL: The infrequency of hypercalcemia in
sarcoidosis. Clin Res 21: 63, 1973.

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PRESENTATIONS

1. Fifth International Sarcoidosis Conference, Prague, 1969; British Thoracic and Tuberculosis Society, Cambridge, England, 1969: Total and ultra-filtrable serum calcium and magnesium in sarcoidosis.
2. Fifth International Sarcoidosis Conference, Prague, 1969: Serum Immuno-Globulins in sarcoidosis.
3. Eastern Section-American Federation for Clinical Research, Washington, D. C., 1969: Hypercalcemia and sarcoidosis.
4. District of Columbia Thoracic Society Symposium on Sarcoidosis, Washington, D. C., 1970: Immunology of sarcoidosis.
5. Southern Section-American Federation for Clinical Research, New Orleans, 1971: Relationship of Kveim reaction to lymphadenopathy in sarcoidosis.
6. American College of Physicians Regional Session, Honolulu, 1971: Kveim antigen and sarcoidosis -- Clinical and Experimental Features.
7. American College of Physicians Regional Session, Honolulu, 1972: Newer immunological aspects of sarcoidosis.

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SECTION 12B

BERNARD WILLIAM JANICKI, Ph.D.

PERSONAL

REDACTED

EDUCATION

University of Delaware

REDACTED

University of Delaware

, The George Washington University

PROFESSIONAL EXPERIENCE

February 1954 to June 1955, University of Delaware, Research Assistant

September 1955 to present, Veterans Administration Hospital, Washington, D.C., Research Microbiologist, currently Chief of the Pulmonary Immunology Research Laboratory

PROFESSIONAL AFFILIATIONS

Scientific Organizations

REDACTED

Academic

The American University, Division of Sciences and Mathematics, Biology Department Thesis Advisory Committee, 1963

Graduate School, United States Department of Agriculture, Biological Sciences Department, Teaching Staff (Immunology), 1964

Georgetown University Schools of Medicine and Dentistry, Lecturer in Microbiology, 1965

University of Maryland, Lecturer in Microbiology, Department of Microbiology, 1969; Associate Member of the Graduate Faculty, 1970

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Other

Consultant, National Institute of Allergy and Infectious Diseases, National Institutes of Health; serving as a member of the Tuberculosis Panel of the U.S.-Japan Cooperative Medical Science Program and Chairman of the Panel's Committee for a Collaborative Study of Tuberculin Antigens, 1969

RECOGNITIONS

Election to Beta Beta Beta, national honor society for Biological Sciences

Election to full membership in the Society of Sigma Xi

Listing in "American Men of Science"

RESEARCH INTERESTS

Basic aspects of host-parasite relationships with emphasis on acquired resistance of the host, especially immunity and delayed hypersensitivity

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BIBLIOGRAPHY

BERNARD WILLIAM JANICKI, Ph.D.

THESES

- Janicki, B.W.: Some aspects of the mechanism of penicillin resistance in pathogenic strains of Micrococcus pyogenes var. aureus. M.A. Thesis, University of Delaware, Newark, Delaware, 1955.
- Janicki, B.W.: A study of the in vitro tuberculin lysis of leukocytes from tuberculin-hypersensitive guinea pigs. Ph.D. Thesis, The George Washington University, Washington, D.C., 1960.

PUBLICATIONS

1. Patnode, R.A., Hudgins, P.C. and Janicki, B.W.: Studies on the effect of Triton (WR-1339) on guinea pig tissues.
1. Lipide chemistry of lung, liver, spleen, adrenals, and blood. J. Exp. Med. 107: 33-41, 1958.
2. Janicki, B.W.: Circulating levels of the "plasma factor" responsible for in vitro leukocyte cytolysis during sensitization of guinea pigs with Mycobacterium tuberculosis. Amer. Rev. Tuberc. 79: 244-245, 1959.
3. Janicki, B.W. and Patnode, R.A.: Enzymatic determination of in vitro lysis of leukocytes by tuberculin. Proc. Soc. Exp. Biol. & Med. 102: 311-314, 1959.
4. Janicki, B.W. and Patnode, R.A.: Studies on the effect of Triton (WR-1339) on the in vitro tuberculin lysis of leukocytes from tuberculin-hypersensitive guinea pigs. Tuberculology 19: 37-43, 1960.
5. Leahy, W.V.C., Janicki, B.W. and McNickle, T.F.: Effect of Triton WR-1339 on thyroid activity of normal guinea pigs. Amer. J. Physiol. 201: 827-829, 1961.
6. Janicki, B.W. and Patnode, R.A.: Increase in circulating lysozyme-like enzyme following sensitization of guinea pigs with Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 83: 872-877, 1961.
7. Patnode, R.A. and Janicki, B.W.: Leukocytotoxic component of pine pollen. Effect on peripheral blood leukocytes of normal and tuberculin-hypersensitive guinea pigs. Amer. Rev. Resp. Dis. 83: 43-49, 1961.

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8. Janicki, B.W. and Aron, S.A.: Effect of Triton WR-1339 on lipoproteins and lipoprotein lipase of guinea pig plasma. *Proc. Soc. Exp. Biol. & Med.* 109: 507-509, 1962.

9. Janicki, B.W., Aron, S.A. and Bernton, H.W.: Comparison of serological response to pine pollen and tubercle bacilli. *Amer. Rev. Resp. Dis.* 86: 385-390, 1962.

10. Janicki, B.W., Leahy, W.V.C., McNickle, T.F. and Aron, S.A.: Effect of Triton WR-1339 on absorption and fate of I-131 oleic acid in normal guinea pigs. *Amer. J. Physiol.* 202: 367-369, 1962.

11. Janicki, B.W. and Patnode, R.A.: Comparison of in vitro leukocytolysis produced with PPD and with fractions from culture filtrate of Mycobacterium tuberculosis. *Amer. Rev. Resp. Dis.* 87: 748-752, 1963.

12. Miller, G.W. and Janicki, B.W.: Effect of Triton WR-1339 on malignant and normal cell cultures. *Cancer Chemother. Rep.* 39: 1-5, 1964.

13. Rose, M.J., Jr., Aron, S.A. and Janicki, B.W.: Influence of non-ionic surfactants on bacteriophage infections. I. Effect of Triton WR-1339 on T2 coliphage infection. *J. Bact.* 87: 933-938, 1964.

14. Aron, S.A., Rose, M.J., Jr. and Janicki, B.W.: Comparative serologic reactivity of I-131 and I-125 labeled human serum albumin preparations. *Internat. Arch. Allergy* 26: 255-263, 1965.

15. Jorolan, E.P. and Janicki, B.W.: Influence of some non-ionic surfactants on pancreatic lipase activity. *Proc. Soc. Exp. Biol. & Med.* 120: 313-316, 1965.

16. Rose, M.J., Jr., Aron, S.A. and Janicki, B.W.: Effect of various non-ionic surfactants on growth of Escherichia coli. *J. Bact.* 91: 1863-1868, 1966.

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19. Jorolan, E.P. and Janicki, B.W.: Mechanism of hyperlipemia induced by non-ionic surfactants. *Progr. Biochem. Pharmacol.* 2:308-314, 1967.
20. Janicki, B.W. and Aron, S.A.: Induction of tuberculin hypersensitivity and serologic unresponsiveness to tubercle bacilli in newborn and young guinea pigs. *J. Immunol.* 101:121-127, 1968.
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25. Janicki, B.W., Chaparas, S.D., Daniel, T.M., Kubica, G.P., Wright, G.L., Jr., and Yee, G.S.: An evaluation of a reference system for antigens of Mycobacterium tuberculosis. *Amer. Rev. Resp. Dis.* 106:142-147, 1972.
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27. Janicki, B.W., Aron, S.A., Schechter, G.P., and McFarland, W.: Tuberculin antigens active in human lymphocyte blastogenesis. *Proc. Soc. Exp. Biol. & Med.* 141:809-813, 1972.
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29. Janicki, B.W., Aron, S.A., and Berson, A.S.: Technical factors affecting an immunoelectrophoretic reference system for analysis of mycobacterial antigens. *Appl. Microbiol.* 25:130-134, 1973.
30. Janicki, B.W., Good, R.C., Minden, P., Affronti, L.F., and Hymes, W.F.: Immune responses in rhesus monkeys after Bacillus Calmette-Guerin vaccination and aerosol challenge with Mycobacterium tuberculosis. *Amer. Rev. Resp. Dis.* 107:359-366, 1973.

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ABSTRACTS

1. Janicki, B.W. and Patnode, R.A.: The relationship of serum levels of lysozyme-like enzyme to sensitization of guinea pigs with M. tuberculosis. Amer. Rev. Tuberc. 79: 838, 1959.
2. Janicki, B.W.: Skin reactions and in vitro leukocytolysis with fractions from culture filtrate of M. tuberculosis in guinea pigs sensitized with heat-killed tubercle bacilli. Fed. Proc. 19: 211, 1960.
3. Janicki, B.W. and Patnode, R.A.: Comparison of in vitro leukocytolysis produced with PPD and with polysaccharide I fraction from culture filtrate of Mycobacterium tuberculosis. Bact. Proc. 87, 1960.
4. Janicki, B.W. and Aron, S.A.: Effect of some non-ionic detergents on enzymatic hydrolysis of coconut oil. Fed. Proc. 22: 575, 1963.
5. Rose, M.J., Jr., Curtis, P.R., Aron, S.A. and Janicki, B.W.: Effect of Triton WR-1339 on phage infection of Escherichia coli. Bact. Proc. 1951, 1963.
6. Janicki, B.W., Rose, M.J., Jr. and Aron, S.A.: Influence of a non-ionic surface active agent on coliphage infections. J. Gen. Microbiol. 37: v, 1964.
7. Jorolan, E.P. and Janicki, B.W.: Inhibition of the action of pancreatic lipase on coconut oil (Ediol) by some non-ionic surfactants. Amer. Chem. Soc. Abstr. 48C, 1964.
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20. Janicki, B.W., Aron, S.A., and Raychaudhuri, A.: Mycobacterial antigens active in tuberculin shock. Fed. Proc. 29: 702, 1970.
21. Janicki, B.W., Schechter, G.P. and Schultz, K.E.: Cellular reactivity to tuberculin in immune and unresponsive guinea pigs. Bact. Proc. 106, 1970.
22. Janicki, B.W., Aron, S.A., Schechter, G.P. and McFarland, W.: Tuberculin antigens active in human lymphocyte blastogenesis. Fed. Proc. 30: 465, 1971.
23. Janicki, B.W., Goldstein, R.A. and Aron, S.A.: Studies of the precipitating antibody response in tuberculosis. Amer. Rev. Resp. Dis. 103: 894, 1971.
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25. Goldstein, R.A., Israel, H.L., Janicki, B.W., and Yokoyama, M.: Serum immunoglobulin D levels in sarcoidosis and tuberculosis. Clin. Res. 21:72, 1973.

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14: First year budget

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

Robert A. Goldstein, M.D.
Bernard W. Janicki, Ph.D.
Pulmonary Fellow (to be named)15
30
40

REDACTED

Technical (annual salary plus fringe)

Laboratory Technician, Immunology
(to be named)

100

Laboratory Technician, Histochemistry
(to be named)

100

REDACTED

Sub-Total for A

B. Consumable supplies (by major categories)

Guinea pigs 500
Tissue culture glassware, media & reagents 1,200
Radioisotopes, solubilizer, fluor, & reagents 500
Disposable test tubes, pipettes, syringes, scintillation
vials and other glassware 1,000
Chemicals, stains, buffers & reagents 200
Office supplies, reference materials, etc. 200

Sub-Total for B

3,600

C. Other expenses (itemize)

Illustrations, film, photographs 400
Compensation for healthy volunteers nil
Travel to scientific meetings for
principal investigators 1,000

Sub-Total for C

1,400

Running Total of A + B + C

26,000

D. Permanent equipment (itemize)

NONE

Sub-Total for D

nil

E. Indirect costs (15% of A+B+C)

E

3,900

Total request

29,900

15. Estimated future requirements.

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	3,800	2,400	nil	4,269	32,729
Year 3	REDACTED	4,000	2,500	nil	4,515	34,610

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Basic Institutional Support of Pulmonary Immunology Research Laboratory	Veterans Administration	62,463	7/1/72 - 6/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Basic Institutional Support of Pulmonary Immunology Research Laboratory	Veterans Administration	63,623	7/1/73 - 6/30/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to Medical Research Associates,
Inc.

Mailing address for checks

Suite 511, 1133 15th Street, N.W.
Washington, D.C. 20005

Robert A. Goldstein
Robert A. Goldstein, M.D.

Principal investigator *Bernard W. Janicki*
Bernard W. Janicki, Ph.D.

Typed Name _____

Signature _____ Date 6/21/73

Telephone 202 483-6666 216
Area Code Number Extension

Responsible officer of institution

Typed Name N. Marshall Meyers

Title Fiscal Officer

Signature *N. Marshall Meyers* Date 6/21/73

Telephone R
Area Code Number Extension

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#87681 - HAWSH

1003539621

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

August 3, 1973

Grant Application No. 878R1
PULMONARY

To: The committee comprising Drs. Bing, Loosli, Sommers
and Wyatt

Subject: Paul Hamosh, M.D., Georgetown University, D. C.
First renewal application No. 878R1
"The Effect of Smoking on the 'Small Airways'"

History

Current Grant No. 878 is in the amount of \$26,870, with
priority for two additional years.

Application No. 878R1 requests \$28,265, against the
original estimate for this year of \$17,250. The increase is
defended on page 3a of the application.

Documents Submitted. (attached)

1. Application dated July 26, 1973 with summary
Progress Report for January 1, 1973 through
July 15, 1973.
2. Reprint of article (a.) under item 11 on
page 2 of the application (the other two
papers listed have been provided and will
be forwarded if you request).

Comment

This grantee was recently site-visited by CTR staff.

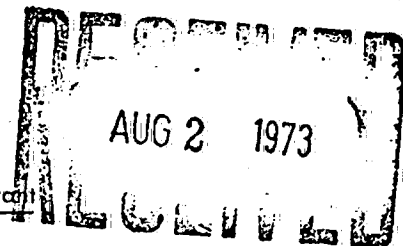
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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885



Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☒Second Renewal ☐

Date: July 26, 1973

1. Principal Investigator (give title and degrees):

Paul Hamosh, M.D., Assistant Professor of Physiology and Biophysics
and Medicine, Georgetown University School of Medicine

2. Institution & address:

Georgetown University School of Medicine
3900 Reservoir Road, N.W.
Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:

Department of Physiology and Biophysics, and Pulmonary Division,
Georgetown University School of Medicine

4. Short title of study:

The Effect of Smoking on the "Small Airways"

5. Proposed renewal date: January 1, 1974

6. How results to date have changed earlier specific research aims:

Earlier specific aims have not been changed. Our own data and recent observations by Bouhuys et al. (1) reinforce the validity of the specific aims: it still remains the demonstration of "reactors" and "non-reactors" to tobacco smoke. The Respiratory Diseases Task Force Report of the National Heart and Lung Institute (2) also essentially subscribes to the approach taken by our proposal.

7. How results to date have changed earlier working hypothesis:

The working hypothesis remains the same, i.e. the existence of two groups of subjects, one group predisposed to develop airway disease from tobacco smoke, whereas the second group is impervious to smoke and "protected" from airway disease. Our first working hypothesis intimated that the difference might be in the innervation of the airways and possible differential innervation of the large airways versus the small airways. Some of our observations are

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7 (continued)

appended (Da Silva, Angelo M.T. and Hamosh, Paul. The effect of smoking a single cigarette on the small airways. J. Appl. Physiol. 34:361-65, 1973). Further study along these lines is warranted especially in view of Bouhuys et al.'s finding (1). However, it would be unwise to postulate that all differences might be due to variations in innervation. Biochemical and immunological components play an equally important, or maybe even more important role. We have recently decided to test the effect of tobacco smoke on metabolism of connecting tissue and surfactant and to quantitate the morphological changes (see pre-proposal by Dr. B. Vidic).

In addition, we discovered the need to add to our protocol the study of regional distribution of inspired gases before and after smoking a cigarette. We wish to introduce ventilatory scans using Xenon¹³³. We developed for this purpose a method enabling us to scan one whole lung and divide it into regions by using a computer technique known as pattern recognition. This consists of electronic scanning of sequential Anger camera slides by a computer (IBM 360/44) connected scanner.

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8. Any additional facilities now required? Describe briefly:

a. In order to improve the quality of our maximum expiratory flow volume curves and especially eliminate the effect of thoracic gas compressions, we would prefer to switch from the present method we use, i.e. the integration of flow to volume from a spirometer (Cardio-Pulmonary Instruments, Co., Model 220) to a Mead-type volume displacement plethysmograph using an electronic circuitry manufactured by Lexington Co.

b. For the safer and more efficient handling of Xenon¹³³ techniques we will need modification of a spirometer.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

Unfortunately we were unable to hire a pulmonary physiology technician with experience even after strenuous efforts at attracting such a person. (It is increaaingly difficult to compete with laboratories delivering clinical service who pay a much more attractive salary and provide a permanent job.) Our present technician whom we had to train from scratch is leaving in September after being admitted to medical school. We have to make a new effort to attract a well-qualified technician and the salary should be commensurate to it.

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

- a. Da Silva, A.M.T. and Hamosh, P. The effect of smoking a single cigarette on the small airways. J. Appl. Physiol. 34:361-365, 1973.
- b. Hamosh, Paul and Da Silva, Angelo M.T. Postural hypoxemia and erythrocytosis due to airway closure at low lung volumes. Am. J. Med. 55:80-85, 1973.
- c. Hamosh, Paul and Da Silva, Angelo M.T. Determination of "closing volume" by using a bolus of room air. Submitted for publication, J. Appl. Physiol. (see appended manuscript).*

* This should be treated as confidential material, since it has not yet been acted upon by the editors.

12. Summary progress report (append in standard form as separate document, unless recently submitted)

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10. The protocol now in use is appended on the following pages. On its completion, a new protocol investigating the effect of cigarette smoke on regional ventilation will go into effect. The method for studying regional ventilation with a very small amount of inhaled Xenon¹³³ has been recently developed by us and it is under trial. Submitting a detailed protocol at this time would be premature.

1003539626

The Dose Dependence of Small Airway Function From Cigarette Smoking

Subjects: Chronic smokers able to "chain smoke" 3 cigarettes in rapid succession without getting "sick" (malaise and tachycardia).

Projected Number of Subjects: 8-12

Methods: The subjects will refrain from smoking for 2 hours before the testing. All testing should be done roughly at the same time of the day. Subjects will have to return 3 times, since only a small number of tests can be performed repeatedly within a limited time span.

1st Series: The following tests will be performed before smoking the first cigarette:

- 1) Thoracic gas volume
- 2) Airway resistance
- 3) Partial expiratory flow volume curve from 50% vital capacity
- 4) Maximum expiratory flow volume curve

These studies are to be repeated after the 1st, 2nd and 3rd cigarettes and then 1 hour following the last cigarette.

2nd Series: The general approach is as above. The following tests will be made:

- 1) An 8-breath nitrogen washout with controlled tidal volume
- 2) "Closing Volume" with bolus method
- 3) Helium partial expiratory flow volume
- 4) Helium maximum expiratory flow volume curve

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3rd Series: The following tests will be made:

- 1) "End tidal" forced expiratory volume at one second (FEV_1)
- 2) Vital capacity
- 3) $FEV_{0.75}$, $FEV_{1.0}$
- 4) MMFR
- 5) MTF
- 6) MVV

4th Series: Selected patients, where warranted, will undergo determination of frequency dependence of compliance and determination of static recoil before and after cigarette smoking.

All subjects will be volunteers from faculty and student ranks.

The procedure is entirely non-invasive and does not pose the slightest risk for the participants.

1003539628

12. Summary Progress Report

This progress report covers the period between January 1, 1973 and July 15, 1973.

a. General: This was a completely new project submitted at a time when the principal investigator changed his affiliation and had to establish a laboratory for investigation of pulmonary physiology from scratch; progress should be viewed in that light.

b. Recruitment of personnel: Mr. Mark Tanker was hired as technician February 1, 1973. He graduated from college with a major in biology. We trained him and presently he is capable of executing all the tests which are part of our protocol.

Mrs. Carole Martin who also worked in the past with us was hired as part-time secretary.

c. Facilities: We have established an all-round pulmonary function laboratory. We have done this mainly from three sources:

(1) Equipment belonging to us through the courtesy of the Veterans Administration with which organization we remain to be affiliated;

(2) From grants provided by the institution mainly with teaching as the primary purpose;

(3) From monies provided by the Council for Tobacco Research and other granting agencies.

d. Experimental Data: Since actual data gathering started only in mid-April obviously the results are not significant at present to draw firm conclusions.

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(1) The Maximum Expiratory Flow Volume (MEFV) curve: We have extended our observations that the mid-section of the MEFV curve is depressed after smoking a cigarette. We have some preliminary observations that this effect might be dose-dependent, i.e. smoking several cigarettes in succession will further decrease the mid-expiratory portion (MEF_{50} of the MEFV curve). We have also started experimenting with Partial Expiratory Flow Volume (PEFV) curves where the subject is inhaling to 60% of vital capacity and then the PEFV curve is analyzed. There is some evidence at present that the PEFV curve might be a more useful index than the MEFV curve.

(2) In the constant search for less complicated and invasive methods we have explored the total respiratory resistance method by the forced oscillation technique as a substitute for the determination of airway resistance by body plethysmography and found that this method indeed is as sensitive to the acute effect of smoking as is airway resistance determined by body plethysmography. We also explored in a number of subjects the "end-tidal" forced expiratory volume presented recently by Dr. Lim at the Federation of American Societies for Experimental Biology meeting (3). This method shows great promise and might ultimately supplant MEF_{50} as a rapid and simple screening method capable of using simple spirometry rather than sophisticated flow-volume tracings.

(3) We have done further work on closing volumes (see appendix). This measurement as performed by the single breath nitrogen washout proved to be not sensitive enough to pick up

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subtle changes after smoking a cigarette. We have devised a bolus method which enhances the resolution of phase IV of closing volume method. The standard error of estimate is much lower with this method, and we hope to be able to detect small changes with a sufficient degree of reliability.

(4) We have applied for the use of blocking agents and marihuana to explore their interaction with tobacco smoke and to use them in "provocation" tests (see original application).

In Summary: Considering the short time available until presentation of this report we have:

- a. Significantly improved and simplified our methodology;
- b. Obtained some preliminary results suggesting the correctness of our working hypothesis;
- c. Developed new methods for testing closing volume and regional ventilation.

REFERENCES:

- (1) C.A. Mitchell, E. Zuskin and A. Bouhuys. The effect of cigarette smoke and β -adrenergic antagonists on small airways. Abstract presented at Annual Meeting of American Thoracic Society, May 21-23, 1973.
- (2) Respiratory Diseases: Task Force Report on Problems, Research Approaches and Needs. The Lung Program, National Heart and Lung Institute, 1972. DHEW Publication No. (NIH) 73-432.
- (3) Thomas P.K. Lim. Detection of early airway obstruction. Fed. Proc. 32:415 (abs), 1973.

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Remarks Pertaining to Budgetary Changes

This budget is higher than the projected one and is about at the level of the first year funding. The increase is due to:

1. An unanticipated need to stipulate partial salary for the principal investigator as a result of changing academic realities (\$2,600);
2. The unexpected increase in salary requirements for qualified personnel (\$2,500 over projected);
3. An increase in the cost of supplies (\$500) because of galloping inflation;
4. An increase in the other expenses (\$500) due to increased cost of publication and cost of other related services;
5. \$4,000 for permanent equipment consisting mostly of electronics and modifications of existing equipment to give us the capability of expanding into new areas of methodology required by recent discoveries in the field.

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")

% time

Amount

Professional (give % time of investigator(s)
even if no salary requested)

Paul Hamosh, M.D.*

10

\$2,942 (10%)

Hall G. Canter, M.D.

5

none

Technical *

Research Assistant (Pulmonary
Technician) to be hired

100

\$12,000.

Carole Martin (Secretary)

40

2,658

* Including 11% fringe benefits

Sub-Total for A

\$17,600.

B. Consumable supplies (by major categories)

Gases, chemicals etc.

1,000.

Recording paper, radioactive gases

500.

Sub-Total for B

1,500.

C. Other expenses (itemize)

Travel

750.

Publications

500.

Computer time (data processing)

250.

Consultation (electronics)

500.

Sub-Total for C

2,000.

Running Total of A + B + C

21,100.

D. Permanent equipment (itemize)

Special electronics for volume plethysmograph
(Lexington Co.)

2,500.

Modification of spirometer for Xenon¹³³ work
(to be incorporated as permanent equipment)

1,500.

Sub-Total for D

4,000.

E

3,165.

E. Indirect costs (15% of A+B+C)

Total request

\$28,265.

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14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Surfactant metabolism as a function of ventilation	Washington Heart Assoc. 3287-785	\$7528.	1/1/73 - 12/31/73
2. Effect of mechanical stress on the elastic properties of the lung in papain induced emphysema	GRS-NIH 3302-113 (through own institution)	\$2500.	1/1/73 - 12/31/73
3. Quantitation of lung cancer on chest films by computer	Contract with the Veterans Administration	\$10,800.	1/1/73 - 12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Computerized Chest X-ray followup in cancer therapy	National Cancer Institute	\$80,460.	1/1/74 - 12/31/76
2. Pediatric lung development program project	National Heart and Lung Institute	approx. 200,000.	7/1/74 - 6/30/79

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Paul Hamosh, M.D.

Signature *Paul Hamosh* Date 7/26/73

Telephone (202) 625-7548

Area Code Number Extension

Responsible officer of institution

Typed Name Guerry R. Smith

Acting
Title Administrator, Sponsored Programs

Signature *Guerry R. Smith* Date 7/31/73

Telephone (202) 625-3151

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Checks payable to

Georgetown University

Mailing address for check: Sam A. Kimble
Georgetown University
37th and O Streets, N.W.
Washington, D.C. 20007

1003539634

Effect of smoking a single cigarette on the "small airways"

ANGELO M. T. DA SILVA AND PAUL HAMOSH

Veterans Administration Hospital and George Washington University, Washington, D.C.

DA SILVA, ANGELO M. T., AND PAUL HAMOSH. *Effect of smoking a single cigarette on the "small airways."* J. Appl. Physiol. 34(3): 361-365. 1973.—We studied the effect of smoking one cigarette on lung function in 21 healthy subjects. Airway resistance measured by body plethysmography increased in 19 patients. "Closing volume," breath-by-breath nitrogen washout and frequency dependence of dynamic compliance (in 7 patients) were not significantly changed after smoking a cigarette. The maximum expiratory flow at 50% of the vital capacity (MEF_{50}) decreased significantly. These later measurements are currently used for assessment of the function of "small airways" (less than 2 mm in diameter). These findings indicate that smoking a cigarette does not increase "small airway" resistance measurably, when procedures not requiring maximum expiratory flow are used. On the other hand, the decrease of MEF_{50} after a maximum expiratory flow-volume maneuver indicates that airways upstream from the flow limiting segment have increased resistance after smoking a cigarette, since no changes in the elastic properties of the lung were demonstrated.

airway resistance; compliance; "closing volume"; flow-volume curve

SMOKING A CIGARETTE increases airway resistance (R_{aw}) (2, 3, 6, 8, 21, 23, 25, 26, 27, 31). This response is mediated by the vagus and suppressed by isuprel and atropine (23, 27, 31). Most studies have been done before the importance of the "small airways" was fully appreciated (13). The "small airways" contribute only 10 to 30 per cent to the total airway resistance and therefore constitute a "silent area" of the lung (16, 20, 30). Determination of the frequency dependence of dynamic compliance (30), "closing volume" (1, 10) and analysis of the maximum expiratory flow volume curve (4) are methods recently introduced to assess "small airway" function. We report the acute effect of cigarette smoke on these measurements.

MATERIALS AND METHODS

Twenty-one subjects were studied (Table 1). Of these, 17 were healthy hospital employees, the rest were outpatients in an alcoholic clinic with no evidence of organic disease. The smokers abstained from cigarettes for two hours prior to the measurements. Thoracic gas volume (V_{tg}) at functional residual capacity (FRC) and the R_{aw} were measured by the method of DuBois et al. (11, 12) using a constant-volume plethysmograph (Warren E. Collins, Inc., Braintree, Mass.).

The "closing volume" (CV) was determined by the single-breath nitrogen washout method (1). After completion of this procedure maximum expiratory flow-volume curves ($MEFV$) were obtained in a volume displacement plethysmograph (Med-Science Electronics, St. Louis, Mo.). The flow was measured with a Fleisch pneumotachograph and a Sanborn 270 differential pressure transducer. The volume signal from the plethysmograph and the flow signal were displayed, respectively, on the horizontal and vertical axes of a storage oscilloscope (Tektronix, Inc., 564B) and photographed. The maximum expiratory flows at the moments when 50% of the vital capacity had been expelled (MEF_{50}) and when 75% had been expelled (MEF_{75}) were read directly from the flow-volume curve. All measurements were made five times and averaged.

Following these procedures, the subjects smoked a non-filter cigarette containing 25 mg tar and 1.5 mg nicotine per g of cigarette (9) in approximately 5 min. Procedures were repeated immediately after the subject finished smoking.

On a separate occasion breath-by-breath nitrogen washout was measured before and after smoking. Lung compliance was measured in seven subjects at different respiratory frequencies (26). Transpulmonic pressure was measured by an esophageal balloon (22) and lung volume by the volume displacement body plethysmograph. Statistical analysis was done using the paired t test.

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RESULTS

The clinical data on the experimental subjects is summarized in Table 1. The age ranged from 25 to 49 with a mean of 33.3 years. Nine out of the twenty-one were active smokers at the time of the study. Only three subjects had never smoked. Nine subjects quit smoking between 1 and 10 years prior to the time of investigation. Five subjects fulfilled the criteria for chronic bronchitis (7).

The results of the respiratory function tests are summarized in Table 2. The vital capacity did not change significantly after smoking. The FRC showed only very small changes. The "closing volume" was determined in 18 subjects and did not show significant changes. Airway resistance increased in 19 of the 21 subjects and the change was statistically highly significant ($P < 0.001$). Conductance and specific conductance both decreased significantly. Since there was not significant increase in FRC after smoking we did not find the specific conductance a more useful expression than conductance itself. Maximum expiratory flow

TABLE 1. Clinical information on 21 subjects

Subj	Age, yr	Wt, kg	Smoking History				Clinical Signs		
			At present	If quit, when? yr	Cigarettes/day	Duration, yr	Cough	Sputum	Duration
NN	31	69	Never						
SR	31	69	Quit	3	20	9			
SL	32	75	Quit	10	40	7			
GG	28	70	Quit	4	10	3			
LJ	31	82	Never						
ME	34	89	Yes		40	17			
DA	33	75	Quit	2	15	15			
HP	40	72	Quit	4	20	17			
SS	31	91	Quit	4	40	10			
NR	31	75	Quit	3	20	13			
CL	32	58	Quit	1	20	12			
PJ	29	77	Yes		20	7			
JE	32	72	Never						
AO	30	64	Quit	3	20	10			
CR	45	75	Yes		40	25	+	+	More than 2 yr
RT	40	73	Yes		40	12	+	+	More than 2 yr
OA	49	86	Yes		50	35	+	+	More than 2 yr
SJ	37	84	Yes		20	19	+	+	Less than 2 yr
GP	25	65	Yes		30	10	+	+	More than 2 yr
MR	26	69	Yes		20	7	+	+	Less than 2 yr
RH	33	56	Yes		30	13	+	+	More than 2 yr
Mean	33.33	73.76							
± SD	±5.91	±9.39							

TABLE 2. Pulmonary function tests before and after smoking a cigarette

Subj	VC, liters		FRC, liters		CV, % of VC		Raw, cm H ₂ O/liter per sec		Gaw, liter/sec per cm H ₂ O		SGaw, liter/sec per cm H ₂ O/liter lung vol		MEF, liter/sec		MEF ₅₀ , liter/sec		MEF ₇₅ , liter/sec		Cdyn, liter/cm H ₂ O	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
NN	5.0	5.0	3.66	3.70	7.5	8.6	1.06	1.27	0.95	0.79	0.26	0.21	8.8	8.6	6.3	5.4	3.3	3.0		
SR	3.9	4.0	2.43	2.56	11.5	11.1	1.26	1.33	0.79	0.75	0.33	0.29	11.0	10.5	6.6	6.0	3.3	3.3		
SL	4.7	4.6	2.49	2.42	11.0	10.6	1.79	1.79	0.56	0.56	0.22	0.23	8.6	8.7	5.3	4.5	2.1	2.0	0.20	0.19
GG	4.6	4.5	3.79	3.75	12.5	11.3	1.27	1.38	0.79	0.72	0.21	0.19	9.9	9.1	4.8	4.5	2.4	2.4	0.21	0.20
LJ	4.2	4.1	2.27	2.15	12.0	11.3	1.32	1.48	0.76	0.67	0.33	0.31	11.5	11.5	5.7	4.8	2.2	2.8	0.19	0.19
ME	4.4	4.1	3.49	3.61	13.2	13.5	1.16	1.12	0.86	0.89	0.25	0.25	11.0	10.7	6.7	6.1	1.6	1.8		
DA	5.0	5.0	4.73	4.36	10.6	10.7	1.06	1.34	0.94	0.75	0.20	0.17	11.0	10.5	5.8	5.7	2.8	3.0	0.20	0.20
HP	4.6	4.5	3.18	3.03	15.3	15.3	0.86	0.93	1.17	1.07	0.37	0.35	9.0	9.1	6.0	6.0	2.4	2.5		
SS	5.0	5.0	3.29	3.01	12.6	15.5	1.26	1.62	0.80	0.62	0.24	0.21	8.7	8.7	5.0	4.6	2.1	2.1		
NR	4.6	4.2	3.66	3.66	8.5	7.1	1.10	1.14	0.91	0.88	0.25	0.24	7.5	7.5	4.9	5.1	2.2	2.5		
PJ	4.5	4.4	2.66	2.57			1.36	1.51	0.73	0.66	0.27	0.25	9.6	8.5	6.3	5.7	3.0	2.7		
CL	4.0	4.0	3.78	3.62	14.3	15.8	1.25	1.48	0.80	0.67	0.21	0.18	7.2	6.6	5.7	5.1	3.5	2.7		
JE	4.2	4.3	4.54	4.58			1.47	1.35	0.68	0.74	0.15	0.16	9.6	9.6	6.2	6.0	5.1	5.0		
AO	4.8	4.6	4.63	4.42			1.02	1.16	0.98	0.86	0.21	0.19	10.5	9.6	6.9	6.9	4.1	5.0		
CR	3.2	3.0	2.93	2.96	29.0	27.0	1.70	1.78	0.59	0.56	0.20	0.18	6.0	6.0	3.3	3.3	1.2	1.3		
RT	3.5	3.6	3.74	3.83	23.0	22.0	1.21	1.31	0.83	0.76	0.22	0.20	9.0	8.7	5.1	4.8	2.5	2.1	0.21	0.22
OA	4.6	4.6	3.57	3.61	27.0	25.0	1.24	1.36	0.81	0.73	0.23	0.20	9.0	7.5	7.2	6.7	3.3	3.0		
SJ	3.7	3.6	3.41	3.45	26.0	24.0	1.46	1.65	0.68	0.60	0.20	0.17	9.3	8.1	4.0	4.1	1.4	1.4		
GP	4.9	4.8	3.56	3.62	14.0	13.0	1.25	1.53	0.80	0.65	0.22	0.18	8.2	7.8	3.6	3.4	1.5	1.5	0.18	0.20
MR	5.6	5.8	3.41	3.37	8.5	8.5	1.57	1.83	0.64	0.55	0.19	0.16	8.2	8.1	5.1	4.8	3.0	2.6		
RH	4.9	5.0	3.53	3.66	15.0	15.0	1.42	1.57	0.70	0.64	0.19	0.17	7.5	7.5	4.0	3.7	1.9	1.7	0.22	0.22
Mean	4.47	4.41	3.46	3.42	15.0	14.7	1.29	1.42	0.79	0.72	0.24	0.21	9.1	8.7	5.5	5.1	2.6	2.6	0.20	0.20
± SD	0.56	0.60	0.66	0.64	6.59	5.97	0.20	0.22	0.10	0.10	0.05	0.05	1.41	1.37	1.09	1.0	0.94	0.94	0.01	0.01
Mean % change					-1.17		+10.93		-9.49		-9.3		-4.15		-6.07		-0.12		+1.55	

VC = vital capacity; FRC = functional residual capacity; CV = closing volume; Raw = airway resistance; Gaw = airway conductance; SGaw = specific airway conductance; MEF = maximum expiratory flow; MEF₅₀ = MEF at 50% of vital capacity; MEF₇₅ = MEF at 75% of vital capacity; Cdyn = dynamic compliance. B = before smoking a cigarette; A = after smoking a cigarette.

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showed a mean decrease of 4.15 % but this change was not significant. MEF_{50} decreased by 6.07 % and this change was statistically highly significant ($P < 0.001$). MEF_{75} remained unchanged but tended to be low in patients who fulfilled the clinical criteria for chronic bronchitis. The dynamic compliance was within normal limits in all subjects and did not significantly change after smoking.

The relationship between dynamic lung compliance and frequency of breathing is shown in Figs. 1 and 2. Only subject GP exhibited frequency dependence of compliance. None of the seven subjects showed significant changes in this relationship before and after smoking.

Breath-by-breath nitrogen washout was performed before and after smoking in 17 of the 21 subjects. The results were plotted on semilogarithmic paper. Sixteen out of the seventeen subjects had linear washout curves. Subject GP had a nonlinear curve. Smoking did not affect significantly the time course and linearity of these curves in any of the subjects. The heart rate was measured before and after smoking the cigarette to assess the circulatory effect. Mean heart rate before smoking was 81 beats/min and after smoking 88. Most active smokers did not respond with increase in heart rate, whereas some nonsmokers showed up to 50 % increase in heart rate.

DISCUSSION

The effect of acute inhalation of cigarette smoke on lung volumes and airway resistance has been studied by Nadel

and Comroe (23) and others (8, 25, 26). The results of this study are only different in the magnitude of the changes. An increase in airways resistance was clearly demonstrated, but the changes in FRC were not significant. The smaller changes are probably due to the fact that each determination was preceded by a deep inspiration to insure uniform volume history. Deep inspiration is known to reduce airway resistance (24). The airway resistance increased in 19 of 21 subjects. This increase in airway resistance is probably due to constriction of the large airways (28). The method used for determination of the R_{aw} is not sensitive enough for measurement of "small airway" resistance (13, 16, 30). Direct measurements of small airway resistance in humans is impossible since it requires partitioning of the pressure drop in the airways at a point in the bronchial tree that is inaccessible to direct measurements (13, 16). Retrograde catheterization in dogs has shown that airways less than 2mm in diameter contribute only about twenty percent of the total airway resistance (16). Theoretically, a two-to-three-fold increase in small airway resistance could remain undetected.

Subjects with chronic cough or heavy cigarette smokers have shown frequency dependent dynamic compliance (15, 19, 30), abnormal distribution of air at increasing respiratory frequency (17), abnormal nitrogen washout curves at high respiratory rates (14) and a reduction in mid-expiratory flow (19). Therefore it has been suggested that the earliest pathologic changes in chronic obstructive pulmonary disease occur in these small airways (13).

Dynamic compliance of the lung (C_{dyn}) in normal subjects is independent of the frequency of breathing (30) at least to 80 cycles/min. It has been reported that C_{dyn} becomes frequency dependent in early lung disease before airways resistance and flow rates show impairment (14, 15, 19, 30). In a recent study, chronic smokers showed a larger incidence of frequency dependence of dynamic compliance than nonsmokers (15). The dynamic compliance may become frequency independent again after discontinuing cigarette smoke (15, 19). These observations indicate an early involvement of the small airways in smokers. Our study shows that smoking one cigarette did not change the relationship between dynamic lung compliance and frequency of breathing in seven subjects. A number of mechanisms can be advanced to explain why C_{dyn} did not become frequency dependent. Since the bronchoconstrictor effect of cigarette smoke is related mostly to concentration of particulate matter (8), it is possible that a concentration gradient exists along the bronchial tree and particles do not reach the small airways in concentrations high enough to cause acute constriction. A nonuniform effect of the smoke in different areas of the lung will also be necessary to produce frequency dependence of dynamic compliance. If the effect is small and all airways were affected uniformly, the dynamic compliance should not become frequency dependent. The absence of response might also be a result of collateral ventilation. Dogs have large collateral ventilation and peripheral airway resistance can increase without causing frequency dependence of compliance (29). Man has less collateral ventilation than the dog, but significant variability might exist.

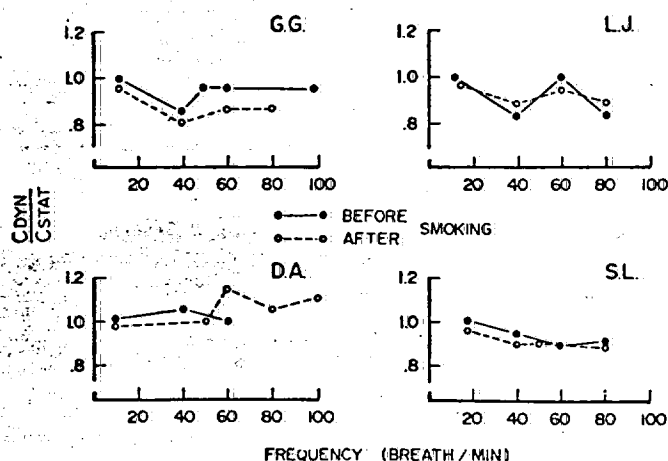


FIG. 1. Relationship between the ratio of dynamic to static compliance and frequency of breathing before and after smoking a cigarette in four nonsmokers.

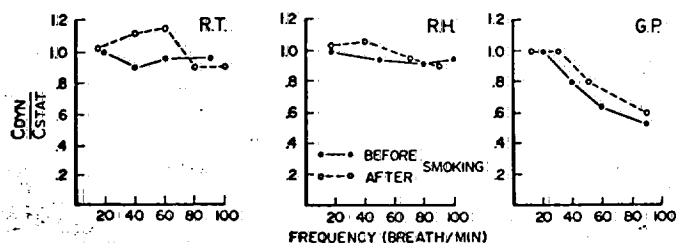


FIG. 2. Relationship between the ratio of dynamic to static compliance and frequency of breathing before and after smoking a cigarette in three smokers.

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Breath-by-breath nitrogen washout was suggested as a very sensitive method for the detection of small airways disease (6). In our subjects only one showed an abnormal washout curve and the same subject had frequency dependence of dynamic compliance. The lack of significant difference before and after smoke indicated that, at least in our hands, this method was not sensitive enough to detect the effects of acute inhalation.

The single-breath nitrogen washout has been recently introduced as a method of measuring "closing volume" (1). Closing volume (CV) represents the lung volume at which the airways in the dependent parts of the lung start closing (1, 5, 10). CV has been found to correlate well with age and smoking history (1, 18). Since airway closure occurs at collapsing transmural pressures, (1, 10) changes in the tonus of the bronchial wall could affect the "closing volume." We found no significant change in closing volume in 18 subjects before and after smoking. This might be due to lack of significant change in the bronchomotor tonus in the bronchi that close at CV. On the other hand, the deep inspiration preceding this procedure might have abolished the bronchoconstrictor effect of the cigarette (24).

The maximum expiratory flow-volume (MEFV) curve shows a drop in midexpiratory range and assumes a concave shape. Since lung compliance remained unchanged in our subjects, reduction of the MEFV₅₀ is probably the result of increased resistance of the upstream segment, because maximum expiratory flow is determined by elastic recoil of the lung and by the resistance of the airways situated upstream from the flow-limiting segment (21). Since the flow-limiting segment is usually in the lobar or segmental bronchi (21), in

addition to the small airways a significant portion of the larger airways is upstream from it. Narrowing of bronchi upstream from the flow-limiting segment and greater than 2 mm in diameter could be responsible for the reduction in flow, rather than the increase in "true" small airway (less than 2 mm in diameter) resistance. This suggestion is further supported by the lack of change in MEFV₇₅. If bronchoconstriction would occur in the "small airways," terminal flow would be equally reduced.

Both the large and small airways are probably affected by the acute inhalation of cigarette smoke causing a decrease of airway conductance and maximum expiratory flow at 50% of the vital capacity. No significant difference in response between smokers, nonsmokers, and subjects with chronic bronchitis was noted. Evidence of increased resistance in the "upstream" segment was found, but whether the "small airways" (less than 2 mm in diameter) are also contributing to the increased resistance has not been conclusively proven. It might be necessary to redefine the demarcation between "small" and "large" airways in more functional terms.

We are indebted to Mrs. C. Martin, Mr. E. J. Morrissey, and Mr. J. L. Landry for technical help.

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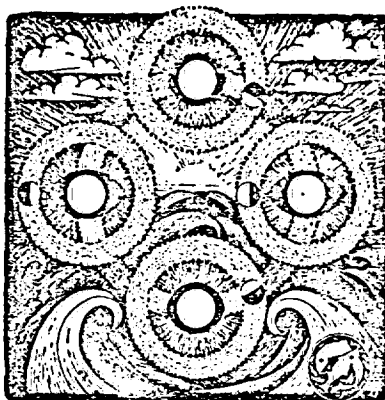
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#914 - IAMM

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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

June 20, 1973

Grant Application No. 914

To: The Committee comprising Drs. Jacobson, Loosli and Wyatt

Subject: Michael E. Lamm, M.D., N.Y.U. Medical Center
New application No. 914
"Immune Mechanisms of Mucous Membranes"

History

This proposal was Case No. 177 and formal application was encouraged by the Executive Committee.*

Application No. 914 requests \$34,672, plus two additional years.

Documents Submitted

Attached is application dated May 17, 1973.

Reprints of the five publications listed on page 3e have been provided, and will be forwarded to you on request.

Comment

As stated on page 5, this is essentially a duplicate of an application now pending at NIH, National Institute of Allergy and Infectious Diseases. Hence the limited legibility of certain pages, which were provided as faint copies.

F.W.N.
F.W.N.

FWN:wg
Encl.

*Dr. Loosli dissented on a ballot received later. He holds that Lamm's proposed study "will add nothing new to an already large body of knowledge", and cites "The Secretary Immunologic System" USDHEW, PHS. December 1969. Supt. of Doc. \$5.25 (paper).

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Dr. Jacobson

Dr. Loosli THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

Dr. Wyatt

110 EAST 50TH STREET

NEW YORK, N. Y. 10022

(212) 421-8885

JUN 11 1973

Application for Research Grant

Date: May 17, 1973

(Use extra pages as needed)

1. Principal Investigator (give title and degrees):

Michael E. Lamm, M.D.

Associate Professor of Pathology

2. Institution & address:

New York University Medical Center

550 First Avenue

New York, N.Y. 10016

3. Department(s) where research will be done or collaboration provided:

Pathology

4. Short title of study:

Immune Mechanisms of Mucous Membranes

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

a. Secretory immunoglobulin A (IgA*), the principal class of antibody in mucous membrane secretions, serves as a first-line defense system at the interface between the interior of the body and the outside world. The unique portion of the secretory IgA molecule is the secretory component (SC) which is not present in any other immunoglobulin, including serum-type IgA. We want to characterize further the SC portion of human exocrine IgA by doing amino acid sequence determinations of selected regions, initially the N-terminus and the regions containing the cysteine residues which form the interchain disulfide bridges. We want to determine the number of disulfide bridges which join SC to the remainder of the molecule and where they are located. For example, is the SC joined to the alpha chain and, if so, what portion?

b. The reason that IgA is the most abundant class of antibody in mucous membrane secretions (whereas in serum it is only a minor component) is the large number of IgA-type plasma cells in mucous membranes compared to cells synthesizing other classes of antibody. We want to gain insights into the mechanisms responsible for the preponderance of IgA-producing immunocytes in mucous membranes.

* Abbreviations: IgA, immunoglobulin A; SC, secretory component; BSC and FSC, bound and free secretory component.

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c. Secretory IgA presents a unique instance among proteins of a molecule of which some components (H, L, and J chains) are synthesized by one type of cell (plasma cell) and another component (SC) by a different type of cell (epithelial cell). We want to investigate the locus where SC is coupled to the remainder of the molecule to give fully assembled secretory IgA, e.g., within, between, or on the surface of the epithelial lining cells of mucous membranes.

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8. Brief statement of working hypothesis:

Secretory IgA plays a key role in host defense, but its chemical structure is much less well understood than that of IgG or IgM, the other principal immunoglobulins. We want to focus on its SC, the portion which renders secretory IgA unique compared to general immunoglobulin structure, in terms of both structure and function. We plan to investigate the hypothesis that SC, which is elaborated by the epithelial lining cells of mucous membranes, serves as a local receptor to capture circulating bone marrow derived (B) lymphocytes that happen to have IgA on their surface. By this means, the preponderance of IgA-type plasma cells in mucous membranes, which is the cellular basis of the secretory IgA system, could be explained.

9. Details of experimental design and procedures (append extra pages as necessary)

A great deal of evidence indicates that local immune mechanisms in mucous membrane secretions are extremely important for defense against a variety of common infectious diseases and furthermore that the antibodies in mucous membrane secretions can be considered as a system which is distinct from circulating antibody (1,2). Thus, in quantitative terms the main immunoglobulin class in secretions is IgA, which is but a minor component of the humoral immunoglobulins where IgG predominates, and in qualitative terms there are differences in the structure of secretory vs. serum IgA. The former occurs as an 11S protein containing 2 four-chain subunits ($\alpha_2\gamma_2$) plus an SC chain and a J chain (3) whereas the latter occurs mostly as a 7S molecule ($\alpha_2\gamma_2$).

We are interested in the SC portion of the secretory IgA molecule because it is distinctive, occurring normally only in secretory IgA, or in a free form in secretions, and being absent from serum. When mixed in vitro with a mixture of immunoglobulins of different classes, it associates preferentially with dimeric IgA (4). (The J chain is not unique to IgA since it occurs in all polymeric immunoglobulins, e.g., polymeric serum-type IgA and IgM). A number of functions have been suggested for the SC portion of secretory IgA such as a role in transporting secretory IgA across epithelial surfaces and protection against proteolysis, but in fact there is presently no compelling evidence for any of the proposals, although there is support for the latter.

Until recently information on the chemical structure of SC has lagged for lack of satisfactory isolation procedures. However, we (5) and Kobayashi (6) have devised methods for purifying human BSC and FSC, and O'Daly and Cebra (7) have been successful with the rabbit counterparts. Our method for isolating BSC is entirely biochemical, the key step being a cyanogen bromide cleavage of the whole secretory IgA molecule, which does not affect the SC since it lacks methionine, whereas Kobayashi's procedure employs an immunoadsorbent. The purification of rabbit BSC has different requirements since unlike the human protein it is non-covalently linked to the remainder of the molecule. Both we and Kobayashi have found human BSC and FSC to be similar in terms of molecular weight, antigenic determinants, and amino acid composition. In addition, we have shown (unpublished) that their tryptic peptide maps and N-terminal amino acid sequences are similar. Thus, it is likely that BSC and FSC are two different forms of the same protein. Whether the FSC has an independent function aside from its role as part of secretory IgA is unknown.

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In addition to the unique features of the secretory IgA molecule, the secretory antibody system presents a number of interesting facets in terms of cellular immunology. For example, secretory IgA is to my knowledge a unique instance of a multi-chained protein of which different portions are synthesized in different cell types, the alpha, L and J chains being secreted by the immunocytes in the mucous membranes whereas the SC is made in the overlying epithelial cells (1,8). The site where SC is complexed to form the complete secretory IgA molecule is not known with certainty, whether between epithelial cells, within epithelial cells, or on the surface of the mucous membrane. Secondly, the reason for the large amount of secretory IgA in mucous membrane secretions can be traced to the abundance of immunocytes in mucous membranes which contain IgA (9-11). In the spleen and peripheral lymph nodes, in contrast, IgA-containing immunocytes are in the minority and most immunocytes contain IgG. The mechanisms which account for this preferential location of IgA-producing immunocytes in mucous membranes are not understood. Two general possibilities come to mind: (1) IgA producers or cells destined to produce IgA preferentially home to mucous membranes or (2) a local inductive influence in the mucous membranes either causes immunocytes preferentially to differentiate into IgA-producers or causes IgA-immunocytes to proliferate more rapidly as compared to other anatomical locations.

There is good reason to believe that the secretory IgA system is of fundamental importance for protecting the body against the entry of potentially pathogenic microorganisms. One of the requisites for understanding this system better is a more complete knowledge of the chemical structure and function of the secretory IgA molecule. To my mind the most interesting portion of this molecule is the SC, which renders it unique when compared with the structure of other immunoglobulins. We have published (5) the only satisfactory procedure for purifying human BSC on a preparative scale, and to my knowledge no other laboratory is actively engaged in the same kind of detailed studies of its chemical structure. (In contrast, the J chain, which we are not studying, is being investigated in a number of laboratories). I think that isolation of BSC via immunoadsorbents, as used by Kobayashi (6) is not so satisfactory for preparative work. This reasoning is consistent with the fact that most of the analytical studies in Kobayashi's paper were done on FSC, and not on BSC which was probably obtained in much more limited quantities. Now that we have developed procedures for isolating BSC, we would like to characterize it more fully. In addition, we want to investigate the mechanisms responsible for the predominance of IgA-immunocytes in mucous membranes and to inquire whether SC plays a role.

Methods of Procedure

1. Amino acid sequence studies

Knowledge of the amino acid sequence of SC is of current interest for two reasons. First, we would like to know if BSC and FSC, which are similar in terms of criteria already studied in our laboratory (size, antigenic determinants, composition and peptide maps) are identical, and the ultimate criterion is, of course, the primary structure. Second, we want to know whether there is any homology to the sequence of immunoglobulin H and L chains. Presumably the evolutionary development of SC is unrelated to that of H and L chains since the pertinent cell types (immunocytes vs. epithelial cells) are so different. However, again the ultimate criterion is the amino acid sequence. Comparison of the sequence of SC with the sequences of other proteins may shed light on its evolutionary origin.

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SC, as we (5) and Kobayashi (6) have shown, has a molecular weight of 71,000 daltons. The largest proteins (immunoglobulin or otherwise) sequenced thus far are a human gamma chain, 53,000 daltons (by Edelman's group) and a mu chain, 70,000 daltons (by Putnam's group). In both cases the proteins, being from patients with multiple myeloma, were available in unlimited amounts and were easily purified by standard techniques. Furthermore, the investigators could be guided by homologies to previously sequenced L chains and portions of H chains. Even so the sequence determinations required several years. In contrast, in our own work on SC we have no myeloma protein sources and only modest supplies of colostrum. We have first to isolate secretory IgA and then from it isolate SC. Also, since human SC is devoid of methionine, it would not be possible to work with cyanogen bromide fragments of the parent protein, a procedure which usually simplifies sequence studies. In conclusion, because of its large size and lack of ready availability in large quantities, I do not believe it will be feasible in the near future to attempt a total sequence of SC.

On the other hand I do believe that SC is a significant and interesting protein and that important information can still be obtained by amino acid sequence studies of selected portions, which we are doing. To my knowledge no other laboratory is engaged in such studies on human SC.

a. N-terminus

We have found that both BSC and FSC are amenable to automated sequence analysis from the N-terminus. (In this regard human SC may be different from the rabbit counterpart in which an N-terminal residue could not be identified (7)). In collaboration with Dr. E.C. Franklin's laboratory (where the automated sequencer is located) we have determined the first 21 residues of human BSC and FSC, which are identical:

Lys-Ser-Pro-Ile-Phe-Gly-Pro-Glu-Glu-Val-Asp-Ser-Val-Glu-Gly-Gly-Ser-Thr-()-Thr-Ser-

This is the only sequence information available on human SC. There are no obvious homologies to H and L chains, which is in keeping with the synthesis of SC by a different cell type, the epithelial cell. We are attempting to extend this N-terminal sequence further into the molecule.

b. Disulfide links to the remainder of secretory IgA molecule

We and others have shown that, unlike rabbit SC, human SC is covalently linked to the remainder of the molecule and can be liberated only after partial or complete reduction. To which chain or chains the SC is joined (e.g., alpha, J or L) and the number of such links are unknown. We want to investigate these questions.

1) Number of interchain disulfide bridges

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SC is isolated after partial reduction and alkylation with radioactive iodoacetic acid. If it is assumed that only interchain disulfide bridges are cleaved under mild conditions, the number of counts incorporated into SC and/or the number of S-carboxymethylcysteine residues per mole after amino acid analysis will reveal the number of interchain disulfide bonds between SC and the remainder of the molecule. It is, of course, possible for labile intrachain bridges to be reduced under mild conditions, which would lead to an overestimation, but in general the assumption holds. Certainly all the interchain bridges will be split because mild

reduction is sufficient to lift one SC. Our initial results show that 2 disulfide bridges form the interchain connecting links to SC.

2) Sequences around the interchain disulfide bridges of SC

SC is isolated after mild reduction and radioactive alkylation with ^{14}C -iodoacetic acid, which marks the interchain cysteines. In order to determine SC preparatory to enzyme digestion, interchain bridges are broken by complete reduction and the sulfhydryl groups of the interchain cysteines are blocked with cold iodoacetamide. The completely reduced and alkylated SC is then digested with enzymes to produce short polypeptides. Initially we shall be studying peptic-tryptic digests (a combination that has proved suitable in the past). The radioactive peptides containing the interchain cysteine residues are isolated by appropriate methods (gel filtration, ion exchange or paper chromatography, or high voltage electrophoresis on paper). The last method works well for small charged peptides, and the extra negative charge conferred on the interchain cysteines by the alkylation with iodoacetic acid proves advantageous. The various steps during the isolation of the radioactive peptides are followed by scintillation counting, and in the case of high-voltage electrophoresis on paper by autoradiography on x-ray film. Purified peptides are sequenced by the dansyl-Edman and/or subtractive Edman techniques.

3) Pairing of the interchain disulfide bridges

The approach discussed above in part 2) is the most sensitive and efficient way to isolate those cysteine peptides of SC which partake in interchain bridges. However, it gives no information on the pairing of these peptides (i.e., to which particular cysteine in the remainder of the secretory IgA molecule is each interchain cysteine of SC joined) because the interchain disulfide bridges are cleaved in the initial mild reduction required to introduce the radiolabel. One approach to this question is to do a cyanogen bromide digestion of the whole secretory IgA molecule (which will not affect SC since it lacks methionine) without prior reduction, and then isolate SC-containing fractions using our specific antisera as guides. The object then is to determine which other portion of the parent molecule is still attached to SC by disulfide bridges. This approach will be simple or complex depending on the location of methionine residues (e.g., in alpha chain) with respect to the disulfide bridge forming the link (e.g., from alpha chain) to SC. Preliminary work indicates that the first methionine from the N-terminus of the alpha chain occurs C-terminal to where SC and L chains join so that SC is part of a large fragment containing much of the alpha chains and L chains.

Two additional approaches can be used to determine the arrangement of the bridges. One is diagonal electrophoresis (12) in which a proteolytic digest of a protein is electrophoresed on paper, after which the disulfide bridges are cleaved directly on the paper by performic acid vapor (oxidation of cystine to cysteic acid). Then a second electrophoresis, under identical conditions to the first, is done in a direction perpendicular to the first dimension. Cysteic acid peptides appear off the "diagonal", their mobility, unlike the other peptides, having been altered by the oxidation, and can be isolated. The two halves of the same disulfide bridge will be located in the same band, and thus the nature of the cysteine pairings can be determined. In this method all the cysteine peptides in the secretory IgA molecule will be affected, and we are mainly concerned with those joining SC to the remainder of the molecule. It should be possible, however, to focus on the appropriate peptides in the diagonal maps because from the studies described in part 2) we would know the electrophoretic mobilities and sequences of the interchain cysteine peptides of SC. If these last can be identified in the diagonal maps, their partners would contain the cysteines to which they had been joined in the native secretory IgA molecule. These latter cysteic acid peptides would then be purified and sequenced.

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In addition to diagonal electrophoresis proteolytic digests of whole secretory IgA could be fractionated by conventional techniques and then provide containing cysteine bridges purified. Among these cysteine peptides would be the cysteine residue identified in part 2). Their partners would contain the cysteine residue elsewhere in the secretory IgA molecule to which they are joined in the native protein.

In our laboratory we thus hope to determine which cysteine residues in BSC participate in interchain bridges and to which cysteines elsewhere in the molecule they are joined. For each partner of an interchain SC cysteine residue we might not know (depending on how the above work turns out) from which portion of which chain (alpha or J) it originated. These questions could be answered by isolating radioactive cysteine peptides from the other component chains of secretory IgA after partial reduction and radioactive alkylation. However, we are not primarily interested in the alpha and J chains, and other investigators, including Frangione and Franklin at this institution, are actively studying these portions of the IgA molecule. Hopefully our data could be meshed with data on cysteine peptides from alpha and J chains obtained by others to answer questions of mutual interest.

2. Histological studies

As mentioned earlier, there is general agreement (e.g., 11,13) mostly from work with fluorescent antisera, that alpha and L chains (i.e., serum-type IgA) are secreted by plasma cells in the lamina propria of mucous membranes and that SC is made by the overlying epithelial cells. However, different workers disagree on the extent of alpha chain staining within epithelial cells. Thus, the actual route of transport to the lumen is still not understood (i.e., between epithelial cells or through epithelial cells) nor is the locus of coupling of SC to the serum-type IgA to yield secretory IgA known (i.e., between or within epithelial cells, or in the lumen).

Poger and I are studying this question in a novel way. In Ouchterlony plates with antisera made against secretory IgA and FSC, we observed reactions of partial identity which showed that SC in secretory IgA and FSC have distinct as well as shared antigens, presumably conformationally dependent. With appropriate insoluble immunoadsorbents we have been able to render anti-FSC entirely specific for FSC (i.e., no reaction with secretory IgA) and anti-BSC entirely specific for the bound conformation (no reaction with FSC). In the former instance anti-FSC is absorbed with secretory IgA, and in the latter instance anti-secretory IgA is absorbed with serum-type IgA, normal serum, and FSC. These absorbed antisera have been fluoresceinated so that we possess reagents specific for SC in its bound vs. free state, and we have been examining human tissue obtained from surgical specimens. We find that FSC occurs in epithelial cells from the Golgi zone (where the most brilliant fluorescence occurs) to the apex, but not at the base. BSC appears to be present only in the apical cytoplasm (Fig. 1). However, with the resolution available in the fluorescent microscope we cannot rule out that BSC merely overlays the apical surface (microvilli) and is not actually intracellular. This distinction will probably require electron microscopic studies, which are planned. (Goblet cells, which others have reported to contain SC, are negative, which we consider to be indicative of the high quality of our reagents.) Alpha chains are distributed exactly the same as BSC (and also, of course, in plasma cells). Our interpretation is that IgA secreted by lamina propria plasma cells passes into the cytoplasm of the overlying epithelial cells where it complexes with FSC. Fully assembled secretory IgA is then elaborated by the epithelial cells.

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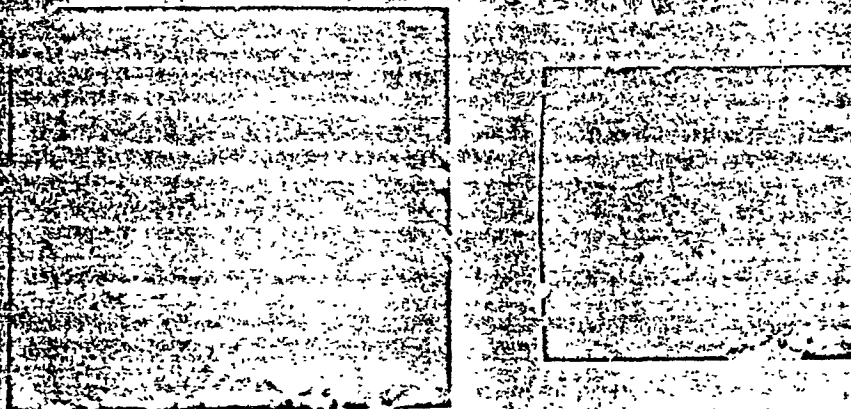


Fig. 1. Fluorescent antibody staining for SC in mucosa. Left--FSC in Golgi and apical portions of epithelial cells. Right--BSC in apices of epithelial cells.

3. Cellular studies

In terms of its cellular components, the secretory immune system has several distinctive characteristics which differentiate it from the more classical humoral antibody system. Consequently, the data now available on the surfaces, intracellular contents, and migratory patterns of B lymphocytes, which have been obtained mostly from spleen, pooled lymph node cells, bone marrow, blood, and thoracic duct lymph, may not hold for the secretory immune system. We therefore want to focus on certain aspects of B lymphocytes as they specifically relate to mucous membranes and for which there are no data in the literature.

It is well known that IgA-containing immunocytes are preponderant in mucous membranes as compared with immunocytes containing other immunoglobulin classes (1, 9-11). Studies designed to explore possible mechanisms responsible for this phenomenon will be carried out in collaboration with Dr. Julia Phillips-Quagliata of our department.

Classes of immunoglobulins are distinguished by differences in their C_H regions, the portions of immunoglobulins which are responsible for their so-called biological properties or effector functions such as complement fixation, sensitization for anaphylaxis, cytophilia, etc. The hypothesis that we want to test is that the C_H regions of immunoglobulins on B lymphocytes influence their migration and homing patterns. Specifically we want to know whether IgA on the surface of certain B lymphocytes is responsible for the predominance in mucous membranes of IgA immunocytes, perhaps because of an interaction with a particular receptor (such as SC, which is synthesized by the lining epithelial cells). Or alternatively could lymphocytes be induced to differentiate into IgA-immunocytes because of an interaction with a local receptor such as SC? We shall use mice because of the availability of inbred strains for cell transfers, well characterized differentiation antigens, and the availability of multiple myeloma proteins.

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a. Nature of surface immunoglobulins on lymphocytes from mesenteric nodes and Peyer's patches

The first question we would like to explore is the nature of the immunoglobulins on the surfaces of lymphocytes in mesenteric nodes and Peyer's patches. We have chosen these sites for our studies of mucous membranes for convenience since bronchial lymph nodes in mice are much smaller and an analogue for Peyer's patches in pulmonary tissue is not well established. There is good evidence that the predominant cell surface immunoglobulin of mouse B lymphocytes from lymph nodes and spleen is IgM (14,15) and that bone marrow cells have surface IgG as well (16). Fixed Peyer's patch cells (rabbit) have been found by immunofluorescence not to contain intracellular immunoglobulin, including IgA (17). However, for studies of surface immunoglobulin viable cells should be used (18), and to my knowledge viable lymphocytes from mesenteric nodes and Peyer's patches have not been critically examined for surface immunoglobulins. We would like to see if perhaps IgA is better represented on such cells. We shall use cytotoxic tests and immunofluorescence and/or autoradiography after incubating cell suspensions with specific radioiodinated (125 I) antisera (19). These studies are underway.

b. Homing experiments

Working with rats, Gowans and Knight (20) showed that large dividing thoracic duct lymphocytes injected intravenously home to intestinal lymphoid tissue. Grisetti *et al.* (21), also with rats, observed a difference in the homing behavior of the large dividing lymphocytes obtained from mesenteric vs. peripheral nodes. The former tended to go to intestinal and the latter to peripheral lymphoid tissues. In studies with rabbits Craig and Cebra (22) demonstrated that lymphoid cells from Peyer's patches can seed the intestinal lymphoid tissue more effectively than cells from peripheral nodes and in addition can proliferate and differentiate into IgA-immunocytes. In the experiments with rats cited above the cells which home to the gut were not studied for their potential to form different classes of immunoglobulin, and in neither species was the nature of the immunoglobulin on the surfaces of the cells which homed to the gut examined.

We have been doing analogous homing experiments in Balb/c mice with lymphoid cells that are labelled with both ^{51}Cr (23) and ^{125}I UDR. The latter labels only large lymphocytes, which when obtained from mesenteric, as compared to peripheral, nodes preferentially home to mesenteric nodes and small intestine. This homing pattern is not so distinct with the ^{51}Cr label. We now want to use combined autoradiography and immunofluorescence (19) to examine whether cells which home to mucous membrane-associated lymphoid tissue are mainly IgA-type lymphocytes in terms of both their cell surface immunoglobulin at the time of transfer and their cell surface and intracellular immunoglobulin after tissue localization. Cell surface immunoglobulin will be studied on live cells in suspension and intracellular immunoglobulin after fixation (18). The population which homes to mucous membrane-associated lymphoid tissue will also be characterized by other means. Thus, before intravenous injection radiolabeled cells will be treated with class specific, anti-kappa, or anti-theta antisera plus complement, or F(ab')_2 fragments of the above without complement, or will be depleted of complement receptor lymphocytes (24). Experiments will also be done with lymphocytes from neonatally thymectomized animals. In addition to ^{125}I UDR and ^3H -thymidine, which label only dividing cells, we shall also use ^3H -uridine and ^{51}Cr which will label most of the cells to be injected. Chromium-labeled cells cannot be followed by autoradiography but offer the advantage of simplicity of counting.

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In addition to efforts to interfere with selected populations of lymphocytes in vivo prior to infection into recipients, attempts to interfere with homing by creating the recipient site will also be made. Thus, if homing to mucosal lymphoid tissues depends on the interaction between exposed Fc regions of cell surface IgA and a receptor, it might be possible to interfere with homing either by passively flooding the recipients with IgA (to compete with cell surface IgA for the putative receptor) or by injecting papain-treated anti-SC antiserum into the recipient in an effort to prevent mucosal membrane SC, postulated to be the receptor, from interacting with cell surface IgA. I am not aware of mouse SC having been purified, and at the moment we have no mouse SC with which to prepare antiserum. It might be easier to purify a small amount of SC from rat, rather than mouse, colostrum and use this as antigen if it cross reacts strongly. As a short-cut we have prepared chicken antiserum against our purified human SC and shown that among mouse proteins in serum and colostrum it is also specific for SC.

Some of the experiments described above are already in progress. Since the homing phenomenon is more apparent among those lymphocytes which are synthesizing DNA ($^{125}\text{IUdR}$ label) as compared to the general population (^{51}Cr label), we also want to do experiments on cells which have been exposed to T cell and/or B cell mitogens (25), which will increase the dividing population.

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Significance

A large body of evidence indicates that secretory immunoglobulins are of particular significance for defense against exogenous infectious agents. Furthermore, the secretory immune system has certain features which set it apart from the humoral immune system and suggest that it be considered separately. Among these novel features is the occurrence of IgA in a special form, secretory IgA, the cellular basis of which is local synthesis within the mucous membrane by two different cell types, immunocytes which make alpha, L and J chains, and epithelial cells which make the SC. The research proposed in this application seeks to enhance our understanding of some of the key features of this secretory immune system. The chemical studies are designed to explore the structure and mode of attachment of the SC component of secretory IgA, the portion of the molecule which is unique in terms of general immunoglobulin structure because it is normally absent from other immunoglobulins, including serum-type IgA, and is synthesized by epithelial cells rather than immunocytes. The studies *in vivo* will focus on the cellular basis for the predominance of IgA in mucous membrane secretions, namely the large numbers of IgA-type immunocytes, which are responsible for the local synthesis of IgA. We hope to gain insights into the mechanisms underlying this phenomenon and will test the hypothesis that SC serves as a local receptor which causes lymphocytes with IgA on their surface to localize in mucous membranes.

A number of different chronic respiratory diseases such as bronchitis, asthma, and emphysema are either infectious in origin or are rendered more serious by secondary infection. In these diseases damage to tissues can be brought about through the direct action of microorganisms or their products or as a result of the reactive inflammatory process on the part of the host, e.g. release of lysosomal enzymes from leukocytes. Autoimmune mechanisms may also play a role. Hopefully, a better understanding of the special features of the secretory immune system will enhance the possibilities for manipulating it so as to improve the treatment and prevention (e.g. by local immunization) of many infectious and perhaps also atopic diseases.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location).

The general facilities of New York University Medical Center are available, including adequate animal quarters. My own laboratory includes 700 square feet of space and the following major equipment: Spinco Model 120C amino acid analyzer, Zeiss PMQ II spectrophotometer, fraction collectors, chromatography cabinet, ovens, high voltage electrophoresis tanks and power supply, Spinco L-2 65 ultracentrifuge, Sorvall RC2B refrigerated centrifuge, Radiometer pH-stat and recorder, and two balances. Additional equipment and facilities are available in other laboratories of the Department of Pathology as may be needed, e.g., electron microscopes, scintillation counters, and a small animal irradiator. Also, I am a joint owner of a Spinco amino acid sequencer and a Hewlett-Packard gas chromatograph which are located in the laboratories of Dr. E.C. Franklin in the Department of Medicine at N.Y.U.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

Attached

13. Publications. (five most recent and pertinent of investigator(s), append list, and provide reprints if available).

Attached

1003539653

CURRICULUM VITAE

Michael Emanuel Lamm

REDACTED

Date and Place of Birth:

REDACTED

Family Status:Education:

REDACTED

Amherst College

University of Rochester School of Medicine, M.D., with honor

Western Reserve University, M.S., Chemistry

Diplomate, American Board of Pathology

Positions Held:

1959-1962

Intern and Assistant Resident in Pathology,
Institute of Pathology, Western Reserve University and
University Hospitals of Cleveland

1962-1964

Senior Assistant Surgeon and Surgeon (Research Associate),
U.S. Public Health Service, Section on Physical Chemistry,
National Institute of Mental Health, Bethesda, Maryland

1964-1968

Assistant Professor of Pathology, New York University
School of Medicine

1968-

Visiting Scientist, Laboratory of R. R. Porter, Dept. of
Biochemistry, University of Oxford

1968-

Associate Professor of Pathology, New York University
School of MedicineMilitary Service:

1962-1964

Commissioned Officer, U.S.P.H.S., Reserve Corps on active duty

Societies:

1959

1959

1966

1967

1967

1967

1967

REDACTED

Honors and Fellowships Received:

1956

National Science Foundation Summer Fellow, Atomic Energy
Project, University of Rochester School of Medicine

1956-1957

Student Fellow in Pathology, University of Rochester
School of Medicine

1960-1962

Resident Training Fellow in Pathology, U.S. Public Health
Service, Institute of Pathology, Western Reserve University
and University Hospitals of Cleveland

1966-

Career Scientist of the Health Research Council of the
City of New York

1972, June

Visiting Professor of Immunology, State University of
Campinas, Brazil.

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CURRICULUM VITAE

Charlotte Cunningham Rundles

Date and Place of Birth:

REDACTED

Education:

REDACTED

Duke University, Durham, N.C.; B.S., Zoology-Chemistry
Columbia University, New York; M.D.

Positions Held:

1972-present

Post-Doctoral Fellow, Department of Pathology, New
York University School of Medicine

1969-1972

Intern and Resident in Medicine, New York University-
Bellevue Medical Center

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3c

Michael E. Lamm, M.D.

CURRICULUM VITAE

Michael McWilliams

Date and Place of Birth:

REDACTED

Education:

REDACTED

University of California, Davis, Calif.; B.S.,
Veterinary Medicine
University of California, Davis, Calif.; D.V.M.,
Veterinary Medicine
- University of California, Davis, Calif.; Ph.D.,
Comparative Pathology

Honors:

Phi Beta Kappa
Phi Zeta

Positions Held:

1971-present

New York University School of Medicine, Post-Doctoral
Fellow, Department of Pathology

1968-1970

Capt. U.S. Air Force, School of Aerospace Med.,
Brooks A.F.B., Texas

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3d

Michael E. Lamm, M.D.

CURRICULUM VITAE

Marshall Poger

REDACTED

Date and Place of Birth:

Education:

REDACTED

Washington University, St. Louis, Mo.; B.A., Physics,
Chemistry

Brandeis University, Boston, Mass., M.A., Chemistry

Univ. of Tennessee, Memphis; M.D.

Positions Held:

1971

1970-71

Resident (Pathology), New York University Medical Center
Intern (Medicine and Pathology), New York University
Medical Center

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13. Five most recent publications:

- 1) Pincus, C.S., Lamm, M.E. and Nussenzweig, V. Regulation of the immune response: suppressive and enhancing effects of passively administered antibody. J. Exp. Med., 133:987, 1971.
- 2) Eden, A., Lamm, M.E. and Nussenzweig, V. Complementarity of H and L chains from anti-hapten antibodies of different classes. J. Immunol., 108:1605, 1972.
- 3) Lamm, M.E. and Greenberg, J. Human secretory component: Comparison of the form occurring in exocrine immunoglobulin A with the free form. Biochemistry, 11:2744, 1972.
- 4) Lamm, M.E. and Frangione, B. Intrachain disulfide bridges of rabbit immunoglobulin light chains of allotypes b4 and b5. Biochem. J., 128:1357, 1972.
- 5) Lamm, M.E., Koo, G.C., Stackpole, C.W. and Hammerling, U. Hapten-conjugated antibodies and visual markers used to label cell surface antigens for electron microscopy: an approach to double labeling. Proc. Nat. Acad. Sci. U.S.A., 69:3732, 1972.

1003539658

Michael E. Lamm, M.D.

14. First year budget.

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s)
even if no salary requested)

	% time
Michael E. Lamm, M.D.	50%
Charlotte Cunningham-Rundles, M.D.	100%
Michael McWilliams, Ph.D.	100%
Marshall Poger, M.D.	50%

Technical

Stephanie Rais (research technician)	100%
Alexandria Warner (laboratory assistant-- glassware washing)	50%
New York Univ. Med. Ctr. fringe benefits	

Sub-Total for A

B. Consumable supplies (by major categories)

colostrum at \$1 per oz.	1,000
mice: 2,000 at \$.80 + 2.5c per day maintenance for 1 mo.	3,100
rabbits: 40 each at \$7 + 34c/day maintenance for 2 mo.	11,096
glass and plasticware	2,000
chemicals, including radiochemicals	3,500
miscellaneous supplies	500

Sub-Total for B

C. Other expenses (itemize)

travel (to attend scientific meetings)	300
service contracts for Spinco ultracentrifuge (L265) and amino acid analyzer (120c)	1,030
miscellaneous repairs and maintenance	300
scientific books and journals related to project	200
duplicating and laundry charges	300

Sub-Total for C

Running Total of A + B + C

D. Permanent equipment (itemize)

Shandon Cyto-Centrifuge (to prepare from small amounts of sample suspensions cell monolayers for microscopic study)	890
Spinco #30 ultracentrifuge rotor (to work up large batches of colostrum)	895

Sub-Total for D

E

E. Indirect costs (15% of A+B+C)

Total request

15. Estimated future requirements.

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	DACTED	11,500	2,230	-	4,533	34,752
Year 3		11,800	2,330	-	4,790	36,723

REDACTED

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5.

Michael E. Lamm, M.D.

16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Studies on Secretory Immunoglobulin	National Institutes of Health (AI-09738)	~\$55,000 per year	

N.B. The present application and the one to the NIH are similar. If both shall be funded, I shall accept only one.

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Y. York University Medical Center
 Attention: Mr. John Ballou
 Mailing address for checks
550 First Avenue
New York, New York 10016

Principal investigator

Typed Name Michael E. Lamm, M.D.
 Signature Michael E. Lamm Date 5/15/73
 Telephone 212 679-3200 X2888
 Area Code Number Extension

Responsible officer of institution

Typed Name Ivan L. Bennett, Jr., M.D.
 Title Director
 Signature Ivan L. Bennett, Jr. Date 5/15/73
 Telephone 212 679-3200 X3977
 Area Code Number Extension

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#741B-LAUMER YNS

1003539661

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

June 22, 1973

Grant Application No. 741B

To: The committee comprising Drs. Gardner, Loosli, and Wyatt

Subject: Joseph M. Lauweryns, M.D., Ph.D., University of Leuven,
Belgium
Continuation application No. 741B
"The Lymphatics of the Lung . . ."

History

This study has been supported by CTR since 1970. The 1973 level of support is \$21,090.

Application No. 741B requests \$24,556. Dr. Lauweryns seems to consider this request for the second year of a three year program, and budgets a third year at \$27,016.

We have no commitment and this request competes as a new application.

Documents Submitted

Attached is an application dated June 2nd, with four addenda. Addendum 4 is a Progress Report, August 1972 - May 1973. Reprints, galley proofs, or manuscripts of recent publications have been submitted, and will be copied for you if you so request.



F.W.N.

FWN:wg
Encl.

1003539662

Comm.
Dr. Gardner
Dr. Loosli
Dr. Wyatt

CHRONIC FULMURARY DISEASES

#7423

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

110 EAST 50TH STREET
NEW YORK, N. Y. 10022
(212) 4218845

Application for Research Grant
(Use extra pages as needed)

JUN 19 1973

Date: June 2nd, 1973.

1. Principal Investigator (give title and degrees).
- Lauweryns, Joseph-M., M.D., Ph.D., Professor Ordinarius in Microscopic Anatomy and Pathology ; Chairman, Principal Investigator.
2. Institution & address:
Experimental Laboratory of Cardiopulmonary and Genital Pathology, Department of Pathology, University of Leuven, 12, Minderbroedersstraat, B - 3000 LEUVEN-BELGIUM.
3. Department(s) where research will be done or collaboration provided:
Experimental Laboratory of Cardiopulmonary and Genital Pathology, department of Pathology, University of Leuven, 12, Minderbroedersstraat, B - 3000 LEUVEN-BELGIUM.
4. Short title of study:
The lymphatics of the lung. Their role in fluid transport and clearance of airborne particulate matter in normal and experimental conditions and in various lung diseases.
5. Proposed starting date. January 1, 1974 (first continuation or renewal application)
6. Estimated time to complete: From January 1, 1973 till December 31st, 1975.
7. Brief description of specific research aims.
Despite our experience in the proposed field of investigation, numerous aspects of the structure and function of the pulmonary lymphatics are still either largely unknown or a matter of considerable controversy in the literature. Each technique of study having its shortcomings, these can only be solved accurately by a further and multidisciplinary investigation of the pulmonary lymphatics which will include various techniques, i.e. - anatomical injection studies, - stereomicroscopical studies, - serial reconstructions, - radiography and microradiography, - histological techniques, - morphometrical techniques, - histochemical techniques, - transmission electron microscopy, freeze-etching electron microscopy and scanning electron microscopy.
During the three years of this research proposal we intend to study the pulmonary lymphatics - with the techniques proposed - along three major lines of investigation:
- (1) normal morphology of the pulmonary lymphatics, - (2) an experimental study of the various morphological factors involved in the clearance and lymphatic drainage of the lung parenchyma, - (3) and morphological studies of the lung lymphatics in various pathological conditions especially in such cases where the formation of lung edema is observed (e.g. neonatal lungs : hyaline membrane disease ; adult lungs : pulmonary edema, especially when associated with chronic respiratory insufficiency as in chronic bronchitis or emphysema, shock lungs (cardiogenic and neurogenic), uremia, drowning..).
Though the lines of investigation are distinct, the study object (i.e. the lymphatics) is identical and the results interrelated.
These studies will necessarily end in important and original contributions which will have many basic and applied results in the structure and function of the normal and diseased lung. It is obvious that these studies are of immediate and relevant importance in biological tobacco research.

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8. Brief statement of working hypothesis:

2.

A combined and multidisciplinary morphological investigation by a team of investigators working closely together since several years, will allow to avoid the shortcomings of each individual technique of investigation in lymphatic research. Studies of pulmonary fluid transport and of airborne particulate matter in normal and diseased lungs and in experimental conditions urgently request a precise and up-to-date knowledge of the pulmonary lymphatics, which is still lacking.

9. Details of experimental design and procedures (append extra pages as necessary)

See separate pages - Addendum 1.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

- All physical facilities are available at our laboratory (as mentioned under item 2), except a scanning electron microscope. As the purchase of a scanning electron microscope has not been funded on the budget of our present CTR-grant (see former application), we carry out this aspect of our work - as earlier - at the University of Ghent, Central Laboratory of Electron microscopy, Chairman Prof. A. LAGASSE, St. Pietersnieuwstraat 41, B - 9000 GHEENT - Belgium.
- Separate list of these physical facilities - see addendum 2

11. Additional facilities required.

- As the proposed purchase of a scanning electron microscope was not funded on our earlier CTR-application, we do propose to continue this aspect of our research program at the University of Ghent (see item 10).
- Hence no additional facilities are requested.

i.e. - Lauweryns, Joseph-M., M.D., Ph.D., principal investigator
 - Boussauw, Luc, Lic. Biol. Sc., full-time research assistant, doctorandus. Co-investigator.
 - Desmecht, Monique, (Mrs. Gombeer), Lic. Biol. Sc., full-time research assistant. Co-investigator.

12. Biographical sketches of investigator(s) and other professional personnel (append):

See separate pages - Addendum 3

13. Publications. (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

Publications and Progress Report - see separate pages - Addendum 4.

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14. First year budget:

*A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

- Lauweryns Joseph

60 %

- Boussauw Luc

90 %

- Desmecht Monique

90 %

R

Technical

- Two laboratory technicians

100 %

(Agnes Thijs ; Ons Stefaan).

R

Sub-Total for A

R

B. Consumable supplies (by major categories)

Animals

2.260

Supplies

10.000

Sub-Total for B

12.260

C. Other expenses (itemize)

Sub-Total for C

none

Running Total of A + B + C

24.556

D. Permanent equipment (itemize)

Sub-Total for D

none

E

none

E. Indirect costs (15% of A+B+C)

Total request

24.556

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	as detailed above					
Year 3	R	13.520				27.016

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16. Other sources of financial support

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Morphological studies of the lung.	University of Leuven	REDACTED	
	J.M. Lauweryns, Professor salary		yearly
	L. Boussauw, assistant, salary		yearly
	M. Desmecht, assistant, salary		early
	Salary of 2 technicians		arly
	Supplies and animals		arly
PENDING OR PLANNED			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
	none		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Joseph M. LAUWERYNS,Signature *J. M. Lauweryns* Date June 2nd, '73.Telephone R Area Code Number Extension

Checks payable to

Prof. P. DE SOMER, Rector of the University.

Mailing address for checks

Dr. P. DE SOMER, Rector, Catholic
University of Leuven,
B - 3000 LEUVEN - BELGIUM

Responsible officer of institution

Typed Name Pieter DE SOMERTitle RectorSignature *P. De Somer* Date June 10th '73Telephone R Area Code Number Extension

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ADDENDUM 1

9. DETAILS OF EXPERIMENTAL DESIGN AND PROCEDURES

1003539668

THE EFFECTS OF THE LYMPHATIC SYSTEM IN LUNG TUMORS
AND CHANGES OF LYMPHATIC FUNCTION IN NORMAL
AND EXPERIMENTAL CONDITIONS AND IN VARIOUS LUNG DISEASES

DETAILS OF EXPERIMENTAL DESIGN AND PROCEDURES. POSTER PLAN

A. INTRODUCTION AND SPECIFIC AIMS

(1) Literature

1.1. As regards the normal morphology of the pulmonary lymphatic system a considerable interest has developed in recent years and much time and efforts have been spent in the study of its structure and function. Especially in the morphological field and following injection studies (Lauweryns et al., publication nr. (26), 1962, (33), 1965, (69), 1970, (89), 1971, Pennell, 1966, Pump, 1970, Trapnell, 1963, Bastianini, 1967, a,b), histological (Lauweryns et al., publication nr. (31), 1963, (34), 1964, (36), 1964, (37), 1965, (46), 1966, (60), 1968, (85), 1970, (86), 1970, (121), 1972, Aminova, 1963, 1967, Grau, 1965, Jdanov, 1969, Karpe, 1965, Kriz, 1970, Oehmke, 1968), histochemical (Borst et al., 1969, Fruschelli, 1967, 1966), radiological (Pennell, 1966, Trapnell, 1963, Lauweryns et al., publication nr. (26), 1962, (34), 1964, (69), 1970, (96), 1971) and especially electron microscopic (Borst et al., 1969, Lauweryns et al., publication nr. (57), 1968, (88), 1970, (96), 1971, (115), 1971, (124), 1972, (125), 1972, Casley-Smith, 1961, 1967, Cliff, 1970, Collan et al., 1970, Fruschelli, 1970, Kato, 1966 a, 1966 b, Klika, 1963, Kriz, 1970, Kühnel, 1966, Leak, 1966, 1967, 1968, 1968, 1970) Oehmke, 1968, Schipp, 1967, 1968, Takada, 1971, Vajda, 1971, Viragh, 1966) investigations of the lymphatic vessels of several body regions, important discoveries have been made, which have led in turn to new ideas and hypothesis concerning the functioning of the lymphatic system. In this way the existence of open junctions (Leak, 1965, 1966, 1968, 1968, 1971, Casley-Smith, 1961, 1967, 1969) and of anchoring filaments (Leak, 1966, 1967, 1968) led to the flap valve concept of the endothelial junctions (Collan, 1970). The absence of a continuous basement membrane and the presence of open junctions and cytoplasmic vesicles (Casley-Smith, 1969) explained the high permeability of the lymphatic endothelium. The presence of filaments within the lymphatic endothelial cells suggests that these cells might have an active and contractile role (Kajno, 1969, Schipp, 1968). The recovery of important amount of tracer proteins (Leak, 1970, 1971, Földi, 1955) in the lymphatic endothelium suggests that these cells might have phagocytotic properties. The ultrastructural aspect and composition of lymphatic valves also raised important ideas about their functioning (Lauweryns et al., (85), 1970, (104), 1970, (96), 1971, (115), 1971, Vajda and Tomcsik, 1971).

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Our investigations were directed almost exclusively towards the physiology of the lung for several reasons. First of all, we were struck by the contradiction between the idea that the pulmonary tissue was a "dry" tissue in which no lymph was formed, as a consequence of the low pressure of the pulmonary circulation (Fick, 1964) on the one hand, and the very extensive pulmonary lymphatic plexus on the other hand (Lauweryns, publication nr. (35) 1960, (215) 1961).

Secondly we realized the importance of the pulmonary lymphatic system at birth, when the fluid contents of the pulmonary capillaries and alveoli is apparently largely and very quickly removed via the pulmonary lymphatics (Aherne and Davkins, 1964, Boston, 1965, Humphreys et al., 1967). Failure of this removal is somehow related to the hyaline membrane disease (Lauweryns et al., publication nr. (38), 1965, (39), 1965, (56), 1968, (70), 1968, (74), 1968, (76), 1968, (80), 1969, (83), 1970) or the idiopathic respiratory distress^{syndrome} of the newborn, a disease responsible for a large percentage of neonatal deaths (Avery, 1964). Finally the importance of the pulmonary lymphatic system for the efficiency and well functioning of respiration and vice versa is gradually becoming clear (Maier, 1966).

Our efforts were successful and we were able to extend the knowledge obtained mainly from lymphatics from skin and mesenterium (Boret, 1969, Casley-Smith, 1961, 1967, Cliff, 1970, Leak and Burke, 1968, Oclake, 1968, Okuma, 1970, Schipp, 1968) and to compare them to the pulmonary lymphatics; to confirm various aspects of their structure and to contribute with several important discoveries, such as the juxta-alveolar lymphatics and the pericentriolar filamentous bundles (Lauweryns et al., publication nr. (38), 1965, (56), 1968, (57), 1968, (60), 1968, (69), 1970), (70), 1968, (74), 1968, (76), 1968, (80), 1969, (85), 1970, (86), 1970, (104), 1970, (121), 1972, (124), (125), Lauweryns, publication nr. (26), 1962, (31), 1963, (34), 1964, (36), 1964, (37), 1965, (39), 1965, (46), 1966, (58), 1970, (89), 1971, (96), 1971, (115), 1971).

1.2. As regards an experimental approach of the various morphological factors involved in the clearance and drainage of the lung parenchyma, we have summarized the available data of the literature on the hereby included scheme 1.

A simple glance at this scheme¹ reveals that :

- no morphological data are available on the precise mechanisms of lymphatic lung drainage (except for the light optical tracer studies of Casarett et al., 1964), and that

- much contradiction exists as to the results obtained, e.g. in the all or not phagocytotic properties of the type I and type II alveolar epithelial cells, even when the investigators have used the same tracer substance (see scheme 1 for thorotrast, carbon and chinese India Ink studies).

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SCHEM 1

BRINKMAN a.s. 1957	-	dog	bovine protein in saline	intratracheal injection		+		
CO. 1900 and SILVERMAN, 1949	-	rabbit	silicium	intratracheal injection	9 h.			
SEAL and STAFFORD, 1951	-	dog	various ions	intratracheal injection				
COOKE and HARRISON, 1954	+ (LO)	rat	India ink	intratracheal injection	4 h. 24 d.	+	+	+
LOW and SALT- PAJO, 1957	+ (LO)	rat	thorotrast	intratracheal injection	1/2 h.		+	
CLECHING, 1952	+ (LO)	rat	barium-hydrox carbon gold hemoglobin India ink	intratracheal injection	10 min. to 6 w.		+	+
KA. R., 1958	+ (LO)	mouse	India ink	intratracheal injection	1 1/2 h.		+	
POLIGARD a.s. 1959	+ (LO)	rat	silicium	intratracheal injection			+	
KARRER, 1960	+ (LO)	mouse	India ink	intratracheal injection	2 h. & 1 d. 9 d.		+	+
ST. M. a.s. 1957	-	dog	I^{131} albumin- I^{131}	intratracheal injection	1 h. 3144h 1 h. 3144h	+		
CASARIT, 1964	+ (LO)	rat	Polonium-210	inhalation			+	
CASARIT and MILLEY, 1964	+ (LO)	rabbit	$^{210}\text{Po}(\text{OH})_2$ / $^{239}\text{PuO}_2$	inhalation			+	
CASARIT and MILLEY, 1964	+ (LO)	rabbit	$^{210}\text{Po}(\text{OH})_2$ ^{210}Po colloid tagged silver	intratracheal injection	1 & 30 d. 1 & 28 d.		+	
SCHULTZ a.s. 1964	-	isolated lung of dog	albumin- I^{131}	intratracheal injection	1 h. 5 h. 1 h. 24 h.			
LADMAN and FINLEY, 1966	+ (LO)	dog	thorotrast	incubation of alveolar wash	1/2 h.			
ECNSCH a.s. 1967	-	dog	I^{131} -albumin I^{131} -globulin	intratracheal injection	15 m. & 7 d.			
DOVINGUEZ a.s. 1967	-	gained rat dog	albumin polyvinylpyr- rolidone	intratracheal injection	1/2 h 45h 3 & 5 d.	+		
RIDEN, 1967	+ (LO)	mouse	carbon	inhalation	1/2 h.			
CASEY and PHELPS, 1963	+ (LO)	rat	polonium-210	inhalation			+	

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Author(s)	Year	Species	Chemical	Route	Dose	Effect	Notes
CHURCHILL	1968	+	rat	intratracheal	100 mg	+	
CHURCHILL and MAY, 1969	+	rat	intratracheal	100 mg	+		
CHURCHILL	1969	+	rat	intratracheal	100 mg	+	
CHURCHILL and CHENG, 1969	+	rat	intratracheal	100 mg	+		
CHURCHILL, 1970	+	rat	intratracheal	100 mg	+		
CHURCHILL, 1970	+	rat	intratracheal	100 mg	+		
CHURCHILL and FAULKNER, 1970	+	rat	intratracheal	100 mg	+		
CHURCHILL, 1970	+	rat	intratracheal	100 mg	+		
CHURCHILL and ALLEN, 1970	+	rat	intratracheal	100 mg	+		
CHURCHILL and DOMINGUEZ, 1971	+	rat	intratracheal	100 mg	+		
CHURCHILL	1971	+	rat	intratracheal	100 mg	+	
CHURCHILL and KARNOVSKY, 1971	+	rat	intratracheal	100 mg	+		
CHURCHILL and BLOOM, 1972	+	rat	intratracheal	100 mg	+		

. Morph. Invest. : Morphological Investigation

P. Alv. Wall : Permeability of the Alveolar Wall

Leukoc. : Leukocyte

-	-		-				-			
+	+			+						
+	+			+						
	+	-	+		+	+	+			?
-	-									
-	+									
	+									
-	+		+		+		+			
	+									
-	+	-	+				+			
-	+	-	+		+	+	+			

LEGEND

Type II Cell : Type II alveolar epithelial Cell

Type I Cell : Type I alveolar epithelial Cell

Epith. Junct. : Epithelial Junction

Interstitium : Basement Membrane and Interstitium

Conn. Tissue : Connective Tissue Cell

End. EV. : Endothelial Cells of Blood Vessels

End. EV. Junct. : Endothelial Junctions of Blood Vessels

Lum. EV. : Lumen of Blood Vessels

Endoth. Lymph. : Endothelial Cells of Lymphatics

End. L. Junct. : Endothelial Junctions of Lymphatics

Lum. Lym. : Lumen of Lymphatics

Lym. Node : Lymph Node

?^{HE} : not demonstrated ; hypothetically formulated

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About one year ago (see progress report -) we have started an experimental study in this respect (using ferritin as a tracer), and it is obvious (Lauweryns and collaborators, unpublished data) that a careful experimental investigation using various morphological techniques and considering the newest literature data on lung structure and function, will yield new and basic results.

1.3. As regards morphological studies of the lung lymphatics in various pathological conditions of the lung parenchyma, it may be stated that no real data are available in the literature, except for casual annotations and our earlier histological (Lauweryns et al., publication nr. (38), 1965, (39), 1965) and morphometrical (Lauweryns et al., publication nr. (56), 1968, (80), 1969) studies on the lung lymphatics in neonatal hyaline membrane disease (76), 1965, (38), 1970) and in drowning (Lauweryns, (92), 1970).

Still the formation of lung edema occurs daily in medical practice in a large number of patients, in association e.g. with chronic and progressive respiratory insufficiency as in chronic bronchitis or emphysema, shock lungs (cardiogenic and neurogenic), uremia, drowning...

It does not seem believable that (almost) nothing is known about the fine morphology of the lung lymphatics in diseased and edematous lungs.

(2) Aims and rationale

From the foregoing we feel that our studies on the lymphatics of the lung should be carried on, as they are not only basically important but are also directed to understand the diseased lung better.

The aims of the proposed study are indeed :

2.1. A further and thorough morphological investigation of the lymphatic system of normal lungs, i.e.

- High power electron microscopy and enzyme digestion studies of anchoring and endothelial filaments.
- Comparative electron microscopic investigations of capillaries, collecting ducts and conducting channels in various animal species and body localizations.
- Electron microscopic and scanning electron microscopic studies of the "roots" of the pulmonary lymphatic system and the valves.
- The innervation of the lung lymphatics.

2.2. An experimental approach of the various morphological factors (especially the lymphatics) involved in the clearance and drainage of the lung parenchyma of airborne particulate matter.

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2.3. A morphological study of the lymphatic system in various diseases (chronic progressive pulmonary insufficiency in chronic bronchitis or emphysema, acute lungs (neoplastic or non-neoplastic), cancer, etc.).

These three areas 2.1., 2.2. and 2.3. will be investigated in parallel during the three years of our research proposal.

2.1. Morphology of the pulmonary lymphatic system

The most urgent questions to be solved in this field are :

2.1.a. The nature and the function of the anchoring filaments.

As it is up to now virtually impossible to isolate these filaments and to submit them to chemical analysis, their nature and function might perhaps be discovered by a high resolution EM in order to compare them with other well known filaments like actin, myosin, protocollagen, combined with enzyme (hyaluronidase, elastase,...) digestion studies. One may also wonder about the nature and the function of the filaments situated within the lymphatic endothelial cells (Cecio, 1967). In a similar way high power electron microscopy for comparative reasons combined with enzyme digestion methods and the study of their morphological reaction towards pharmacological agents might reveal their nature and function.

2.1.b. The nature and the function of the very recently discovered pericentriolar filamentous bundles (Lauweryns et al., (124), (125), in press). A study of their distribution in different cell types and in various animal species might at least reveal whether they are specific to some animals, to some cell types and might hence suggest possible roles. If they were specific to lymphatic endothelium and present in various species, their role would certainly be related to lymphatic function.

2.1.c. The endings of the pulmonary lymphatic system. Are the terminal divisions of the small lymphatic capillaries (the initial lymphatics, Caeley-Smith, 1961) blind fingerlike projections, on themselves closed loops, or is there fine communication with tissue clefts (Klika, 1968) ? This problem can best be studied by the use of electron microscopic techniques which allow a certain degree of tridimensional visualization and surface view, i.e. freeze-etching and especially scanning electron microscopy.

2.1.d. The pulmonary lymphatic valves. Our graphic reconstructions (Lauweryns et al., publication nr. (85), 1970, (86), 1970, (89), 1971, (96), 1971, (121), 1972) and stereomicroscopic observations (Lauweryns, publication nr. (111), 1971, (115), 1971) of pulmonary lymphatic valves could most adequately be confirmed and extended by scanning electron microscopy of these valves.

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2.1.e. The morphological differences between lymphatic and blood vessels
collecting channels and efferent channels. It is hardly believable that no data
are known concerning this subject. The answer to this question implies a thorough
electron microscopical and histological observation of a large number of lymphatics
in various localisations and in various animals.

2.1.f. The innervation of the lymphatics and their valves.
(Collert, 1967, Kubik, 1955, Melnikova, 1964, Schipp, 1965, Shdanov, 1967,
Vajda, 1966). Here a combined neurohistochemical and electron microscopic investi-
gation will we have executed in other areas (Lauweryns et al., publication nr.
(84), 1969, (106), 1970, (108), 1971, (117), 1972) - will lead to an answer.

Rationale : These various morphological problems are to some extent
related and can also only be accurately solved (Lauweryns et al., publication nr.
(86), 1969, (96), 1971) by a combined and multidisciplinary approach including
various techniques, which are familiar to us, i.e; : - anatomical injection studies,
- serial reconstructions, - radiography and microradiography, - histological tech-
niques, - morphometrical studies, - histochemical techniques and - electron micros-
copy.

All these techniques currently used at our laboratory have indeed their
limitations, and some of them could even produce artefacts. Injection of the lym-
phatics of the pleura i.e. with radioopaque substances followed by radiography, or
with plastic substances followed by corrosion of the tissues, easily causes dis-
ruption of the delicate walls of the smaller lymphatic vessels and filling of tissue
clefts. The injection moreover may inverse valves and hence result in false pictu-
res of lymphatic drainage-pathways. Corrosion casts and radiographs moreover do not
reveal the relationship of the vessels to the surrounding tissues in fine detail.

Histological examination i.e. of non injected lymphatics is hindered by
difficulties in recognizing pulmonary lymphatic capillaries and in differentiating
them with certitude from small blood vessels and tissue clefts. Histochemical methods
to differentiate lymphatic vessels from other structures are not known.

Reconstructions from serial histological sections (Staubesand et al., 1953,
Comparini et al., 1965, Boussauw et al., publication nr. (85), 1970) are subject
to the same difficulties and have moreover the disadvantage of being a time consu-
ming procedure.

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Electronmicroscopy is also limited because only very small areas can be
investigated.

Freeze-etching electron microscopy will also be applied. This technique is
different from traditional electron microscopy because it avoids some chemical
interactions in the tissues, exposes tissue structures in relief and allows the
observation of membrane surfaces "en face". We are thoroughly acquainted with

this technique, but the study does contain information on the lymphatic system (Lauweryns et al., publication nr. (95), 1971, (97), 1971, (100), 1971, (101), submitted). In studying the lymphatics, it still poses one major problem as basal membranes are not visible on freeze-etch electron micrographs (hence hindering the differentiation from lymphatic capillaries with other small vessels or even tissue clefts).

Scanning electron microscopy will also be executed. It will certainly add important information and new, original and unique data to our field of study; indeed it allows the investigation of relatively large samples (as compared to transmission electron microscopy) both at low and higher magnification and with great focal depth. Moreover the peculiar tridimensional "geometrical" structure of the lung seems to be ideally adapted for SEM investigation.

From the limitations and inherent risks of each of these methods, we feel that only a multidisciplinary investigation by applying various methods of investigation can result in a clear, synthetic and true picture of the pulmonary lymphatic system, as already stands out from our earlier work (Lauweryns et al., publication nr. (26), 1962, (31), 1963, (34), 1964, (36), 1964, (37), 1965, (38), 1965, (39), 1965, (46), 1966, (56), 1968, (57), 1968, (60), 1968, (69), 1970, (70), 1968, (74), 1968, (76), 1968, (80), 1969, (84), 1969, (85), 1969, (86), 1970, (88), 1970, (89), 1971, (96), 1971, (104), 1970, (106), 1970, (108), 1971, (111), 1971, (115), 1971, (117), 1972, (121), 1972, (124) & (125) in press).

2.2. Experimental study of the various morphological factors (especially the lymphatics) involved in fluid transport and clearance of particulate matter

Following the pathway(s) which free particles have to follow to reach the lymphatic capillary lumen from the alveolar lumen, the following factors have to be considered: - alveolar macrophages, - alveolar epithelium, - basal membrane(s), - alveolar interstitium with blood capillaries, - lymphatic endothelium.

2.2.a. The alveolar macrophages, whose origin is seriously controverted (Bertalauff and Leblond, 1953, Karrer, 1960, Bowden et al., 1963, Pinkett et al., 1966) do represent an important factor in alveolar clearance (Karrer, 1958, Policard et al., 1959, Faulkner and Esterly, 1969, Sanders and Adey, 1970). They are sometimes aided in their task by leucocytes which may be present in the alveolar lumen as free cells (Gross and Westrick, 1954, Gieseck, 1958, Karrer, 1960, Suzuki and Churg, 1969, Faulkner and Esterly, 1969). After resorption of foreign material the macrophages may (Cole, 1944, Vorwald, 1950, Karrer, 1960, Davies, 1963) or may not (Gross and Hatch, 1962, Hatch and Gross, 1964, Casarett and Milley, 1964) migrate to the interstitium and further on to the lymphatics.

These different aspects should be investigated.

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2.2.b. The phagocytic properties of the alveolar epithelium - as clearly demonstrated in table 1, content of macrophages within the alveolar structure concerning the phagocytotic properties of the alveolar epithelium; this problem is not only important as such, but also because it remains related to the cellular origin of the surfactant (Shacklin, 1957; Mikawa et al., 1965; Niden, 1967; Kohn, 1968; Azopardo and Thurlbeck, 1969; Bannex, 1970 a).

2.2.c. The permeability of the basal membrane(s) which are not observed on freeze-etching electron micrographs (Lauweryns et al., publication nr. (95), 1971, (97), 1971, (100), 1971, (123) submitted; Friederici, 1968), also remains an open question (Schneeberger-Kenley and Karnovsky, 1968).

Precise studies with high resolution electron microscopy on the fate and migration of particles through the interstitium and their interaction with the connective tissue cells have also not been executed.

2.2.d. The way(s) by which particles enter the lymphatic lumen is mainly speculative (Nappleston, 1963). An intercellular transport via typical open junctions is usually accepted (Lauweryns et al., publication nr. (73), 1969), still a transcellular transport may occur as well (Casley-Smith, 1965; Leak and Burke, 1968).

It is also unsettled if the lymphatic endothelial lining cells are capable of storing, digesting or transporting particles?

The same question may be asked concerning the blood capillaries.

Though both vessel systems (blood- and lymph vessels) probably play a role in alveolar clearance, their relative importance remains unsettled (Mayer et al., 1969; Derner, 1970 b, Leak, 1970, 1971).

Rationale : Though using in general terms the same spectrum of techniques, as mentioned under 2.1., this investigation will be mainly electron microscopical.

2.3. The lung lymphatics in various diseases

Being thoroughly acquainted with the lymphatics in the normal adult and infant lung and having approached them already in two diseases (i.e. Hyaline Membrane Disease of the human newborn (Lauweryns et al., publication nr. (38), 1965, (39), 1965, (56), 1968, (76), 1968, (80), 1969, (88), 1970) and drowning (Lauweryns, publication nr. (92), 1970), we propose to investigate them in various diseases (chronic and progressive respiratory insufficiency as in chronic bronchitis or emphysema, shock lungs (cardiogenic and neurogenic), uremia, drowning, hyaline membrane disease, lungs of other neonatal deaths), especially when these are accompanied with edema formation.

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As practically nothing is known about this subject, it will require a considerable task and constitute quite an endeavour ; it is however obvious that we are ideally prepared to investigate this challenging problem, being thoroughly acquainted with the normal anatomy of the lymphatics and being indeed an a pathologist.

Rationale : Gross (including lymphatic injections) and routine microscopic studies (including morphometric pilot studies) of the lungs will be first undertaken. Depending on the obtained results further investigations (transmission electron microscopical, scanning electron microscopical, freeze-etching) will be further undertaken.

b. METHODS OF PROCEDURE

(1) Species to be investigated

1.1. As regards the normal morphology of the pulmonary lymphatic system :

- rabbit (newborn and adult).
- human infant and adult lungs.

We are thoroughly acquainted with rabbit and human lungs, as we are studying them since 1953 (cfr. curriculum vitae).

1.2. As regards the experimental study :

- newborn rabbits.

We are indeed not only thoroughly acquainted with their lungs, but have already undertaken these experiments since one year with satisfactory results.

1.3. As regards the lung lymphatics in various diseases :

There will be no problem of collecting lungs as we have currently about 120 infant and 350 adult postmortems yearly.

As regards electron microscopy, we have no problem to be immediately alerted and do the fixations as soon as possible ; we are indeed immediately informed by the "Newborn premature center", the "High care unit" and the "Clinic for chronic lung diseases". The efficiency of this delicate cooperation has been demonstrated in our earlier electron microscopical studies of "Hyaline Membrane Disease" and human "Lung Lymphatics" in normal lungs (cfr. publications - curriculum vitae).

It is clear from the above that we will have no problems to harvest the lungs needed for our investigation.

(2) Techniques to be applied to :

2.1. Normal morphology of the pulmonary lymphatic system :

The techniques to be applied are all familiar to us (enzyme digestion excepted). They are especially :

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- high power electron microscopy (problems 2.1.b ; 2.1.c ; 2.1.d ; 2.1.e).
- high power electron microscopy and computerized digitalization (problems 2.1.b ; 2.1.c ; 2.1.d ; 2.1.e).
- freeze-etching electron microscopy (problems 2.1.d ; 2.1.e).
- scanning electron microscopy (problems 2.1.d ; 2.1.e).
- electron microscopy (problems 2.1.b ; 2.1.e).

As regards the innervation of the pulmonary lymphatics, will be studied by the classical histological techniques of silver impregnation (Gordon-W. Campbell), by histochemical studies on cholinesterases (method of Loelle, modified by Gerebázeff (1959) and by freeze-drying-fluorescent studies on catecholamines (method of Falck, 1962, 1965). We are thoroughly acquainted with these techniques (cfr. Lauweryns et al., publication nr. (53), 1967, (64), 1969, (84), 1969, (103), 1970, (117), 1972).

For a distinction of cholinergic and adrenergic nerve endings we will apply the methods of Richardson (1966), De Robertis and Pellegrino, Wood and Barnett, (1964). Here also high resolution electron microscopy is best applied.

2.2. Experimental study :

- This will be the continuation of our study, started about one year ago. Under slight anesthesia, a tracer (0,05 cc) will be intratracheally instilled. Instillation causes indeed much less tissue disturbance or damage than injection. The amount of tracer introduced in the lungs by this procedure is also constant ; this is not true in cases of intranasal instillation.

- The animals are killed at various intervals after the instillation (from 15 min. to 24 days) and a careful electron microscopical investigation of both lungs carried out.

- Morphometric studies will be carried out to estimate the removal of the tracer and its fine localization in the lung.

- As tracer we will first use a colloidal solution of ferritin (≈ 100 Å 110 Å, MW ± 465000 , 2 Å crystalline, cadmium free, N.B.C., Cleveland) ; Indeed ferritin is not only a widely used and efficient tracer (biological protein, electrondense, non-toxic, Bruns and Palade, 1968) but has also never been used in an analogous study.

- To check the probable influence of the physico-chemical properties of the tracer ferritin, we will next use a totally different tracer, i.e. a carbon suspension (C11/1431 a; Günther Wagner, Hanover) whose composition has been described by Biozzi et al., (1953).

- Both series of results will be compared.

- After these studies using ferritin and a carbon suspension, other tracers could be used, if necessary (i.e. horseradish peroxidase ; thorotrast ; colloidal gold ; etc).

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2.3. The lymphatics in various diseases :

As this constitutes an entirely new investigation with no reliable literature data available, we will apply the whole spectrum of morphological techniques which we have used earlier to study the lymphatics in normal lungs. Indeed and as explained in Rationale 2.1., each technique having its shortcomings, a combined and multidisciplinary approach is inevitable including various methods, which are familiar to us : - anatomical injection studies, - serial reconstruction, - radiography and microradiography, - histological techniques, - morphometrical techniques, - histochemical techniques, - transmission electron microscopy, - freeze-etching electron microscopy and scanning electron microscopy.

The "pilot approach" will include detailed gross and microscopic studies, combined with some injection studies and elementary morphometry.

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 ACTA ANAT., 1967, 67 : 369-386.
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 ARCH. INTERN. MED., 1970, 125 : 450-463.
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 LAB. INVEST., 1968, 18 : 555.

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AM. J. ANAT., 1970, 53 : 77-907.
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GEN. RESP. PHYS. 1970, 1951, 9 : 356.
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AM. J. ANAT., 1971, 152 : 207-215.
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ACTA KARB. ACAD. SCI. HUNG., 1966, 19 : 157-203.
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ACTA KARB., 1971, 73 : 521-531.
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PHYSIOLOGIE, 1966, 19 : 51-53.
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BRIT. J. EXP. PATH., 1966, 47 : 553-567.
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AM. J. PHYSIOL., 1959, 216 : 715-727.

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ADDENDUM 2

10. SEPARATE LIST OF PHYSICAL FACILITIES AVAILABLE

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List of physical facilities available

- a) for histology :
 - all routine equipment : drying, covers, rotary microtome Spencer, microtome for large sections Leica Jung, 3 binocular microscopes, 2 stereomicroscopes.
- b) for radiography :
 - radiographical apparatus Balteau with image intensifier.
 - microradiographical apparatus Balteodyn, Balteau-General Electric.
- c) for histochemistry :
 - cryostat Pearce.
 - freeze-drying apparatus Pearce.
- d) for morphometry :
 - one microscope with lateral projection arm.
 - one Olivetti calculator.
- e) for electron microscopy :
 - one ultramicrotome Porter Blum MT-2.
 - one automatic ultramicrotome Reichert.
 - one vacuum-coating apparatus Edwards.
 - dark room facilities.
 - one freeze-etching ultramicrotome Balzers.
 - one electron microscope Zeiss Em 9 (resolution ± 12 Å).
 - one electron microscope Philips EM 300 (resolution ± 3 Å).

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ADDENDUM 3

12. BIOGRAPHICAL SKETCHES

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BIOGRAPHICAL NOTES

- Joseph M. LAURENUS, principal investigator.
- Title and present appointment : M.D., Ph.D. (19), Professor and Chairman of the department of pathology and microscopic anatomy, The Vrije Universiteit, Katholieke Universiteit Leuven-Netherlands, 12, Minderbroedersstraat, B - 3000 LEEUVEN - Belgium.
- Birthdate : R
- Place of Birth : R
- Nationality R
- Family Status : R
- Former Instruction, Degrees, Research Activities and Appointments :
 - Primary School R and Greco-Latin Humanities (R :
 - Notre-Dame College - Ostend - Belgium and
 - St.- Joseph College - Tielt - Belgium.
 - Official Degrees (Præsumptus and first award of diverse local, provincial and public state examinations).
 - Katholieke Universiteit - Leuven - Faculty of Medicine
 - 12, Minderbroedersstraat
 - B - 3000 LEEUVEN - Belgium.
- 1. Doctor in Medicine ("M.D.")
 - 1951-52 : First Candidature Medicine - July - Magna cum laude
 - 1952-53 : Second " " - July - Cum laude
 - 1953-54 : Third " " - July - Magna cum laude
 - 1954-55 : First Doctorate " " - July - Cum laude
 - 1955-56 : Second " " - July - Magna cum laude
 - 1956-57 : Third " " - July - Magna cum laude
 - 1957-58 : Fourth " " - July - Magna cum laude
- 2. Complémentary Degree of Candidate in Biological Sciences (part.) 1954-55.
- 3. Agrégé de l'Enseignement Supérieur (Ph.D.) (16/05/1962) :
 - Publication of an aggregation thesis, entitled : "De longvaten : hun rol en architectoniek bij de longontplooiing" ; Arsclia, 1962, 302 pg. followed by its public defense (11/04/1962) and
 - a public lecture in the University Promotion Hall : "De anatomopathologie van de long in verband met de perinatale mortaliteit" (16/05/1962).
- 4. "Aspirant" to the "National Belgian Research Funds" 1960-1963.

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5. Fellow of the Association of Specialists in "Pulmonology" (Clinical Pathology-Section of International Congress) since 1953.
6. Doctor (1952-1957) and Senior Lecturer (Professor extraordinary) (1957-) in histology and pathology at the Faculty of Medicine of the Katholieke Universiteit - Leuven, Netherlands (K.U.L.) since 1952 ; Director of the Department of Histopathology and of the Experimental Laboratory for Cardiopulmonary and Genital Pathology which I founded in 1963 in the Vascular Institute. My special fields of interest are indeed cardiopulmonary and genital pathology. The experimental laboratory belongs to the department of Histopathology.

Scientific Institutions

I have been working at the Department of Pathology B of the University of Louvain (Dir. : Prof. P. BRUSSELMANS) since 1953 as a student in medicine. From then onward, I have been interested in pulmonary morphology. My first investigations were an experimental and pathological study of the human's lung expansion at birth and of general perinatal mortality and pathology.

Becoming doctor in medicine in 1958, I was appointed "research assistant" in morphology by the University of Louvain and, while working at the same laboratory, put an end to my previous investigation and became "first lauréat" (section of morphology) at the public state competition of the "Bourses de Voyage" 1959, with a "Bijdrage tot het omvormingsmechanisme in de menselijke long bij de geboorte" and in which I stressed for the first time that a "haemodynamic disturbance" could occur in the hyaline membrane disease of the newborn. In 1958, I joined also the Staff of the Laboratory of Anatomy (Prof. G. VAN DER SCHUEREN) and of the Laboratory of Gynaecologic Physiopathology (Prof. H. RENAIER) and have been working there till 1962.

At the laboratory of anatomy, I gained the necessary experience for angio-architectonic studies ; at the laboratory of gynaecologic physiopathology, I could perform the routine pathology of all cases of perinatal mortality (some 100 cases/yearly) occurring at the University clinics. In 1960, I became "Aspirant" at the "Fond National Belge de la Recherche Scientifique" for a period of three years.

Besides routine pathology my special fields of post doctoral research and interest were :

- the vasculature of the lung : histological, angioradiological and angiomicroangiological study of pulmonary and bronchial circulation in man and dog.
- experimental and pathological study of the human's lung expansion at birth.

So I became in 1962 "Agréé de l'Enseignement Supérieur", which is a public state and university degree, more or less corresponding to a "Ph.D.". (Publication

of anatomical dissection (thesis, entitled: "De longanae longinae in archa...
bij de longanae longinae", 1877, 1878, 1879, 360 pages, followed by the publication
before the Faculty of Medicine; 1880 is shown in the Examen Roll of the University
"De anatomicopathologie van de long in verband met de pathologische veranderingen").

My military duties were fulfilled in 1959 (July) - 1960 (October) as "sous-
lieutenant médecin de réserve" performing, pathology, at the "Central Laboratory
of the Royal Military Hospital (LGSB), Brussels, Belgium (Head of the Department:
Col. Pradi-homme; Cdt. Delporte).

In 1963, I did - as explained under the item "Scientific trips" - during the
second semester of the academic year, a stay in the USA (especially at the Armed
Forces Institute of Pathology, Washington, D.C.) as "Fellow" of the "World Health
Organization".

Finally, I have been appointed in 1962 "dozent" and in 1967 "gewoon hoogleraar"
(professor ordinarius in histology and pathology at the University of Louvain (Belgium).
As my special fields of interest are "cardiopulmonary and genital", I founded an
"experimental laboratory for cardiopulmonary and genital pathology", at the end of
1963. The main work done in this laboratory is a study of the normal and pathological
vasculature of the lung by means of radiology, microradiology and histology of the
same injected specimens. This work has been supported by a PHS grant HE-00393 (RMD)
from the National Heart Institute.

As I had some highly interesting results on the pulmonary arterial vasculature
in the newborn, I made a short stay at the Research Center for Premature Infants
(Dr. M. KLAUS), Stanford University School of Medicine, Dept. Pediatrics and discus-
sed also these results with Dr. John A. CLEMENTS, Cardiovascular Research Institute,
San Francisco, in October 1965. This stay was supported by the University of Louvain
and partially by the Stanford University.

I was awarded again a fellowship of the W.H.O. in 1967 for visiting several
Universities and research Institutions in the U.S.A. I made a stay of one month at
the department of pulmonary and mediastinal pathology (Dr. S. ROSEN) at the Armed
Forces Institute of Pathology in Washington, D.C., and of two weeks at the research
laboratory of Dr. P. Gruenwald in Baltimore. I made several "guest lectures" (cfr.
personal publications - lectures), i.e. at the National Institute of Neurological
Diseases and Blindness, at the National Heart Institute, at Case-Western Reserve
University (Babies Hospital), at the University of Illinois (Department of Anatomy)
and the University of Miami. I visited several prominent research workers in my
field of study, i.e. Profs. M.E. AVERY and P. GRUENWALD, Baltimore, Prof. J. CLEMENTS,
San Francisco, Prof. M. KLAUS, Cleveland, Prof. J. FAISSEL, Denver, Profs. YANIG

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Since 1957, I have been regularly invited to participate to various scientific meetings in the U.S.A. (1958 : Second International Congress of Zoology, Miami ; 1960 : Mexican Asian Conference on Physiology ; 1969 : Pacific Asian Conference on Physiology ; 1970 : Cardiovascular Research Institute, San Francisco ; 1971 : Visiting Professor at the University of Southern California, Los Angeles.) and to give several guest lectures as detailed further on in my curriculum vitae.

Special Awards :

- "Lauréat" of the "Prix Specia" 1958.
- "Lauréat" of the Belgian Public Health Department "Concours de Courses de Voyage" 1959, with a "Bijdrage tot het ontzorgingsmechanisme in de menselijke long bij de geboorte".
- "First Lauréat" of the "Courses de Voyage Specia 1960".
- "Lauréat" of the "Prix Ruffin Schoekaert" 1962 for neonatal research activities.
- Janet Baldwin Memorial Lecturer, 1969, New York (U.S.A.), Lenox Hill Hospital.
- Amelia Block Memorial Lecturer, 1971, San Francisco (U.S.A.), Mount Zion Hospital.
- Visiting Professor of Pathology, the University of Southern California, School of Medicine, Los Angeles, California, May 1971.
- "Lauréat" of the "Prix Dr. G. Scharelhout - Koettlitz" 1968-1970, at the "Koninklijke Vlaamse Academie voor Geneeskunde van België".
- Visiting Professor of Pathology, Nippon University, Tokyo, Japan, August 1972.
- Award from the "American Heart Association", March 1973, Tucson, Arizona.

Academic and Medical Societies :

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Scientific trips and research stases outside Belgium :

- April 1959 : Paris (France) : Laboratory of Pathology (Head of the Department : Dr. Gricouloff) and "Ecole de Puériculture" de la Faculté de Médecine de Paris (Head : Prof. M. Lelong), Université de Paris.
- October 1960 : Paris (France) : "Ecole de Puériculture de la Faculté de Médecine de Paris (Head : Prof. M. Lelong).
- January 1961 : Vienna - Austria : "Anatomisches Institut der Universität" (Head : Prof. H. von Hayek).
- October 1961 : Paris (France) : "Centre d'Etudes et de Recherches des Charbonnages de France" (Head : Prof. P. Policard) "Ecole de Puériculture de la Faculté de Médecine de Paris" Prof. Sarrut).
- 1963 (January-June) : Stay at the "Armed Forces Institute of Pathology" (Prof. Mostofi, Prof. Mahr, Prof. Helwig) in Washington, D.C., U.S.A. as "Fellow of the World Health Organization". Short visit to the Departments of Pathology of University of Illinois Chicago, Ill., Prof. S.R.M. Reynolds ; Harvard Medical School, Boston, Mass., Profs. Hertig J. Craig and P. Young ; Columbia University, New York, N.Y., Prof. W. Blanc ; Yale University, New Haven, Conn., Prof. A. Liebow ; National Institutes of Health, Bethesda, Md., Prof. Stewart ; Participation to the Annual Congress of the "American Association of Pathologists and Bacteriologists" and "International Academy of Pathology" Cincinnati, 26 April- 1 May, 1963 ;

Participation to the "Second Annual Forum for International Medical Scholars-Care," Washington University, School of Med. of Washington - Virginia.

- 1965 (October) : Short stay at the Stanford University School of Medicine ; Stanford Medical Center ; Department of Pediatrics ; Research Center for Premature Infants ; Dr. Marshall Klaus, Palo Alto, California. Side visit to the Cardiovascular Research Institute, University of California, San Francisco.
- 1967 (August 20 - November 5) : Study visit to the U.S.A. as Fellow of the "World Health Organization" at the Armed Forces Institute of Pathology, Department of Mediastinal and Pulmonary Pathology, Dr. S. Rosen ; side visits to the N.I.H., Bethesda, the Case Western Reserve University, the University of Illinois - Chicago and the Jackson Memorial Hospital - Miami.
- 1968 (March) : Invited by the "American Organization Committee" of the "Second International Congress of Lymphology" (Miami, Florida).
- 1968 (June) : Invited by the "Chronic Respiratory Diseases Control Program of the U.S. Public Health Service" to participate at the "Eleventh Annual Conference on Research in Emphysema" in Aspen, Colorado (June 12 - 15). Presentation on the session of "Cell Biology" of "Neonatal Hyaline Membrane Disease : light and electron microscopical studies".
- 1969 (June) : Invited by the "Chronic Respiratory Diseases Control Program of the U.S. Public Health Service" to participate at the "Twelfth Annual Conference on Research in Emphysema" in Aspen, Colorado (June 11 - 14). Presentation on the session of "Lung Morphology" of a lecture : "The lymphatics of the lung : macroscopical and microscopical studies". First "Janet Baldwin Memorial Lecture", Lenox Hill Hospital, New York (June 2) : "Clinical and Pathologic Aspects of Pulmonary Vasculature." Side visit to the Department of Pathology of McGill University, Montreal.
- 1970 (May) : Research stay at "Cardiovascular Research Institute" of the "University of California Medical Center" at San Francisco. Eight International Congress of the International Academy of Pathology, Mexico City (May 11 - 19). Side visit to the Department of Pathology, Duke University School of Medicine at Durham, North Carolina.

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- 1971 () : Visiting Professor of Pathology at the University of Illinois (Chicago) and the University of Arizona (Tucson).
 He was appointed to "Henry Jacoby Distinguished Lecturer" for 1971 at the Mount Zion Hospital (San Francisco). Participated at the Annual Conference of the American Thoracic Society (Los Angeles) and discussed "A stereomicroscopical study of pulmonary lymphatic values".
- 1972 (August) : - Visiting Professor of Pathology at the Nippon University in Tokyo (Japan).
 We conducted research seminars and held postgraduate lectures on pulmonary and pediatric pathology.
 - Participated at the "4th International Congress for Histochemistry and Cytochemistry", Kyoto (August 21-26).
 - Presentation on the session dedicated to "new methods and techniques" : "The ultrastructure of the lung alveoli using various fixatives and/or Freeze-etching" (in collaboration with Mrs. M. Gombeer Desmecht).
 - Side visits at the universities of Tokyo.
- 1972 (November) : Participant (by invitation) to the "International Conference on Pulmonary Reactions to Organic Materials" New York, U.S.A. November 8-11 1972, organized by "The New York Academy of Sciences" with a contribution on "The Lung lymphatics" (in collab. with J. Baert).
- 1973 (March) : -Invited as "Faculty Member" to deal with the pathology of the lymphatic system at the "Refresher Course in Lymphology", on the "4th International Congress of Lymphology", Tucson (Arizona-USA).
 - As chairman of the Symposium on Heart and Lung Lymphatics, he presented the results of a research project entitled : "The rôle of the lung lymphatics in the transport of intratracheally instilled particles".
 - Finally, he participated as "panel member" at 3 workshops on "Lymphatic Research".
- 1973 (April) : - Participant (by invitation) to the "International Symposium on Microcirculation", Lucerne (Switzerland), April 16-18, 1973, organized by the "European Society of Cardiology" with a contribution on "Morphological Studies of the Blood and Lymphatic Micro-circulation of the Lung".

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1. Lauweryns J. : - Soc. Roy. Belge de Gyn. et d'Obst., BRUXELLES (Belgium), 17/10/1957.
- "La mortalité périnatale (cratologie pathologique du fœtus et du nouveau-né)."
- In collaboration with Bonte M. and Bonte J.
2. Lauweryns J. : - 46th Congress of the "Association des Anatomistes", MONTPELLIER (France), 23-3-1959.
- "Base morphologique de l'érection capillaire du fœtus et du nouveau-né."
- In collaboration with Bonte J.
3. Lauweryns J. : - The eleventh "Journées d'Etudes et d'Informations sur le nouveau-né, le prématuré et le nourrisson." Ecole de Puériculture de la Faculté de Médecine de Paris (France), 17-18 October 1959.
- "Etude morphologique de l'expansion alvéolaire au moment de la naissance."
- In collaboration with Bonte J. and Van Der Schueren G.
4. Lauweryns J. : - 47th Congress of the "Association des Anatomistes", NAPLES (Italy), 26-31 March 1961.
- "Documents radiologiques et histologiques sur la circulation bronchique du chien."
- In collaboration with Van Der Schueren G.
5. Lauweryns J. : - The twelfth "Journées d'Etudes et d'Informations sur le nouveau-né, le prématuré et le nourrisson," Ecole de Puériculture de la Faculté de Médecine de Paris (France), 16-17 October 1961.
- "Le syndrome d'atélectasie pulmonaire secondaire à la naissance, étude morphologique et hémodynamique."
- In collaboration with Bonte J. and Van Der Schueren G.
6. Lauweryns J. : - Soc. Belge de Pédiatrie, LIEGE (Belgium), 29-10-1961.
- "Possibilités anatomo-cliniques dans le diagnostic des affections respiratoires du nouveau-né."
- In collaboration with Eggermont E.
7. Lauweryns J. : - Soc. R. Belge de Gyn. et d'Obst., BRUSSELS (Belgium) 01-1962.
- "Les pneumonies dans la période périnatale. Etude anatomo-clinique."
- In collaboration with Eggermont E.

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8. Lauweryns J. : - Soc. Roy. Polpe de Cyn. et d'Hist., BRUSSELS (Belgium)
- Symposium on the "mortalité périnatale d'étiologie obscure"
"Hémorragies pulmonaires et mortalité périnatale."
- In collaboration with Fosse J., Fosse J., Fosse J. and
Candona J.
9. Lauweryns J. : - Soc. Roy. Polpe de Cyn. et d'Hist., BRUSSELS (Belgium)
14-4-1962.
- Symposium on the "mortalité périnatale d'étiologie obscure"
"Hémorragies pulmonaires et mortalité périnatale."
- In collaboration with Eggermont E.
10. Lauweryns J. : - 48th Congress of the "Association des Anatomistes", TOULOUSE
(France), 15-19 April 1962.
- "Documents radiologiques et microradiologiques sur la circula-
tion bronchique et pulmonaire,"
- In collaboration with Van Der Schueren G.
11. Lauweryns J. : - "American Registry of Pathology", WASHINGTON, D.C. - U.S.A.,
25-4-1963.
- "The radiologic appearance of the vessels of the lung."
12. Lauweryns J. : - "Cardiology Clinic, Walter Reed Army Medical Center",
WASHINGTON, D.C. - U.S.A., 26-4-1963.
- "The pulmonary circulation."
13. Lauweryns J. : - "Armed Forces Institute of Pathology", WASHINGTON, D.C. - U.S.A.,
12-6-1963.
- "The vasculature of the lung."
14. Lauweryns J. : - Second European Congress of Anatomy, BRUSSELS (Belgium)
1-6th September 1963 - Exposé principal (par invitation)
(Rapporteur).
- "L'angioarchitecture du Poumon."
15. Lauweryns J. : - "Société Anatomique de Paris, PARIS (France) 23-4-1964.
- "La vascularisation pulmonaire."
16. Lauweryns J. : - "Fifth International Academy of Pathology Meeting," LONDON
(Great Britain), 21-27 June, 1964.
- "The bronchial circulation and its relationship with the pul-
monary circulation on the normal dog's lung."
17. Lauweryns J. : - 49^e Réunion de l'Association des Anatomistes, MADRID (Spain),
6-10 September, 1964.
- "Les lymphatiques du poumon néonatal normal et pathologique
(atélectasie secondaire avec membranes hyalines). Description
d'une nouvelle technique et résultats."

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18. Lauweryns J. : - 15^e Journées d'Etudes du nouveau-né, du prématuré et du nourrisson, PARIS (France), 19-20 October 1964.
 - "Essai anatomo-clinique de 35 cas à évolution fatale d'atélectasie pulmonaire méconariale secondaire d'origine obstructive."
 - In collaboration with Eggermont E., Van den Priecker A. and Denys P.
19. Lauweryns J. : - "Paediatric Pathology Society, the 1965 Meeting, EXETER, 14-16 October 1965.
 - "Neonatal hyaline membrane disease."
20. Lauweryns J. : - Pathology seminar, Stanford University, Medical Center, P.O. BOX 4310, ALTO, California - U.S.A., 21-10-1965. Guest Lecture.
 - "A pathological and experimental study of the lymphatics and the vasculature in the respiratory distress syndrome."
21. Lauweryns J. : - "Studiedag betreffende het probleem van de perinatale mortaliteit", Koninklijke Belgische Maatschappij voor Gynaecologie en Verloskunde, de Hogere Medische Raad van het Nationaal Werk voor Kindervelzijn, en de Koninklijke Belgische Maatschappij voor Paediatric, BRUSSELS (Belgium), 13-11-1965.
 - "Perinatal Mortality."
 - In collaboration with Eggermont E., Van den Driessche A., De Coninck Cl., Denys P. and Renaeer H.
22. Lauweryns J. : - "Tweede Nederlandstalig Symposium van het Belgisch Genootschap voor Heelkunde", Main Subject : Pathology of the oesophagus, BRUSSELS (Belgium), 14-11-1965.
 - In collaboration with Morelle J. and Denys P.
23. Lauweryns J. : - Les "Treizièmes Journées d'Etudes et d'Informations sur le nouveau-né, le prématuré et le nourrisson", 15-16-October 1962, PARIS, (France).
 - "La microangiopathie thrombotique du nourrisson."
 - In collaboration with Denys P., Corbeel L. and Van den Driessche A.
24. Lauweryns J. : - De "Vereniging der Nederlandse Gynaecologen en Verloskundigen", 15-12-1962, LIER (Belgium).
 - "Pulmonale afwijkingen en perinatale mortaliteit."

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- "Pathologie des membranes et du placenta pendant la grossesse normale et pathologique"
- In collaboration with Legendre E., Van den Driessche A. and Denys P.
- 26. Lauweryns J. : - Société Royale Belge de Gynécologie et d'Obstétrique, 5-12-1965, BRUSSELS (Belgium).
 - "Deux cas de listériose foeto-placentaire."
 - In collaboration with Knaeer H., Huyghebaert C. and De Brynes P.
- 27. Lauweryns J. : - IInd Symposium on Steroid Hormones, "Androgens in normal and pathological conditions", 17-19 June 1965, GENT (Belgium).
 - Dysandrogenic forms of male hypogonadism."
 - In collaboration with Steeno O. and De Voer P.
- 28. Lauweryns J. : - Société Belge de Pédiatrie en Nederlandse Vereniging voor Kindergeneeskunde, 4-4-1965, LIÈGE (Belgium).
 - "Les hépatites congénitales à caractère familial. Description et individualisation d'une variété nouvelle."
 - In collaboration with Denys P., Desmet V. and Dechamps L.
- 29. Lauweryns J. : - Paediatric Pathology Society, the 1965 Meeting, 14-16 October 1965, EDINBURGH (Great-Britain).
 - "Neonatal hyaline membrane disease."
- 30. Lauweryns J. : - Pathology Seminar, Stanford University, Medical Center, PALO ALTO, California - U.S.A., 21-10-1965 - Guest Lecture.
 - "A pathological and experimental study of the lymphatics and the vasculature in the respiratory distress syndrome."
- 31. Lauweryns J. : - "Studiedag betreffende het probleem van de perinatale mortaliteit", Koninklijke Belgische Maatschappij voor Gynaecologie en Verloskunde, de Hogere Medische Raad van het Nationaal Werk voor Kinderwelzijn en de Koninklijke Belgische Maatschappij voor Paediatric, 13-11-1965, BRUSSELS (Belgium).
 - "Pathologische Anatomie in verband met de perinatale sterfte."
 - In collaboration with Eggermont E., Van Den Driessche A., De Coninck Cl., Denys P. and Renner H.
- 32. Lauweryns J. : - "Tweede Nederlandstalig Symposium van het Belgisch Genootschap voor Heelkunde", gewijd aan de Pathologie van de slokdarm, 14-11-1965, BRUSSELS (Belgium).
 - "Pulmonale verwickelingen bij slokdarmatresie : hun mogelijke genesis en hun verloop."
 - In collaboration with Morelle J. and Denys P.

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34. Lauweryns J. : - Internationaal pneumologie colloquium, 18-19-1966, GENEVE (Suisse).
 - "Desquamatieve interstitiële pneumonie."
 - In collaboration with Van der Linde L. and De Winter C.
 - In collaboration with Cosmans J., Gyselen A., Afschrift M. and Roelandt J.
35. Lauweryns J. : - 12th Annual Meeting of the Pediatric Pathology Society, 21-22 October 1966, LONDON (Great-Britain).
 - "The vasculature of the lung in the neonatal respiratory distress syndrome."
 - In collaboration with Lerut T. and De Coninck Cl.
36. Lauweryns J. : - Société de Pathologie Thoracique du Nord, 23-10-1966, LILLE (France).
 - "A propos de deux cas de maladie des colombophiles (pigeon breeder's disease)"
 - In collaboration with Eyckmans L., Cosmans A. and Wildiers J.
37. Lauweryns J. : - Belgische Vereniging voor Pathologische Ontleedkunde, 19-11-1966, BRUSSELS (Belgium).
 - "Desquamatieve interstitiële pneumonie."
 - In collaboration with Cosmans J., Gyselen A. and Afschrift M.
38. Lauweryns J. : - Belgische Vereniging voor Pathologische Ontleedkunde, 19-11-1966, BRUSSELS (Belgium).
 - "Pigeon breeder's disease."
 In collaboration with Eyckmans L., Cosmans J., Gyselen A. and Wildiers L.
39. Lauweryns J. : - 52^e Réunion de l'Association des Anatomistes, 2-6 April 1967, CRÉCY (PARIS) (France).
 - "L'ultrastructure des vaisseaux lymphatiques pulmonaires."
 - In collaboration with Boussauw L.
40. Lauweryns J. : - Studiedag van de Belgische Vereniging voor Pathologische Ontleedkunde, 22-4-1967, BRUSSELS (Belgium).
 - "De pulmonale hyaline membraanziekte van de menselijke pasgeborene."
 - Reporter General.
41. Lauweryns J. : - Studiedag van de Belgische Vereniging voor Pathologische Ontleedkunde, 22-4-1967, BRUSSELS (Belgium).
 - "Transpositie der grote bloedvaten met pulmonale recirculatie."
 - In collaboration with Bourgeois H. and Van Der Hauwaert L.

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42. Lauweryns J. : - "Recherche sur les modifications structurales de l'endothélium vasculaire par microscopie électronique" (Belgium).
- "The pulmonary lymphatics."
- In collaboration with Mart A., Boussauw L. and Claessens R.
43. Lauweryns J. : - Annual Meeting of the Association of European Pediatric Cardiologists, 10-13 May 1967, ROMA (Italy).
- "Ischemic heart failure in infants."
- In collaboration with Van der Lauwert L. and Boussauw L.
44. Lauweryns J. : - Colloque de Bruxelles de la Société Belge de Microscopie Electronique de la Société Française de Microscopie Electronique, 22-24 May 1967, BRUSSELS (Belgium).
- "Evaluation au microscope électronique de l'imprégnation neuveuse pulmonaire au tétraoxyde d'osmium-iodure (méthode de Champy-Maillet)."
- In collaboration with Feuskens J.
45. Lauweryns J. : - "Pneumologie - Colloquium" van de Belgische Vereniging voor de Wetenschappelijke studie van de Tuberculose en de Pneumologie, 1-6-1967, MONT-GODINNE (Belgium).
- "Abnormale helderheid van een long."
- In collaboration with Afschrift H., Lacquet A., Cosmans J. and Gyselen A.
46. Lauweryns J. : - "Cardiovascular Research Seminar", Armed Forces Institute of Pathology, 31-8-1967, WASHINGTON (D.C.) - U.S.A.
- "The electron microscopy of the vascular wall."
47. Lauweryns J. : - 13th Tagung der deutscher Gesellschaft für Elektronenmikroskopie 17-21 September 1967, MÜNCHEN (Germany).
- "The ultrastructure of the pulmonary lymphatics."
- In collaboration with Boussauw L.
48. Lauweryns J. : - "Guest Lecture" - "National Institute of Neurological Diseases and Blindness", 19-9-1967, BETHESDA (Md.) - U.S.A., National Institutes of Health.
- "An electron microscopical evaluation of the osmic-acid zinc iodide technique (method of Champy-Maillet) on pulmonary tissue."
49. Lauweryns J. : - "Guest Lecture" - "National Heart Institute", 20-9-1967, BETHESDA (Md) - U.S.A., National Institutes of Health.
- "Pathological and experimental studies of the alveolar wall, the pulmonary lymphatics and vasculature in the respiratory distress syndrome."

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50. Lauweryns J. : - "Guest Lecture" - Department of Anatomy, University of Illinois, Chicago, Illinois (U.S.A.)
- "Hyaline membrane disease of the newborn infant."
51. Lauweryns J. : - "Guest Lecture" - Radiology and Chest Disease Department, Case Western Reserve University, 9-10-1967, CLEVELAND (Ohio) - U.S.A.
- "Hyaline membrane disease of the newborn infant."
52. Lauweryns J. : - "Guest Lecture" - Department of Anatomy-University of Illinois, 12-10-1967, CHICAGO (Ill.) - U.S.A.
- "The lymphatics of the lung."
53. Lauweryns J. : - "Guest Lecture" - University of Miami School of Medicine, 1-11-1967, MIAMI (Fl.) - U.S.A.
- "Pulmonary angiography."
54. Lauweryns J. : - "Guest Lecture" - Department of Radiology, Jackson Memorial Hospital, 1-11-1967, MIAMI (Fl.) - U.S.A.
- "Problems in lymphatic research."
55. Lauweryns J. : - Het vierde Belgisch Nationaal Congres van Pneumologie en Fysicologie en de Belgische Vereniging voor de Wetenschappelijke studie der Tuberculose en der Pneumologie, November 13-16, 1967, BRUSSELS (Belgium).
- "Asbestlichaampjes in longen van lijken." **1003539709**
- In collaboration with H. Van de Voorde.
56. Lauweryns J. : - Meeting of the "Belgische Vereniging voor Electronenmicroscopie" 9-2-1967, LOUVAIN (chairman : J.M. Lauweryns).
- "Electronenmicroscopische studie van de hyaline membraanziekte."
- In collaboration with N. Bourgeois, M.R. Van Hanne and L. Boussauw.
57. Lauweryns J. : - Second International Congress of Lymphology, March 15-20, 1965, MIAMI (Fl.) - U.S.A. (by invitation).
- "Radiological and histological studies of the pleural lymphatics."
- In collaboration with Paert A.L.
58. Lauweryns J. : - Second International Congress of Lymphology, March 15-20, 1965, MIAMI, (Fl.) - U.S.A. (by invitation).
- "Lung changes in the rabbit after oil embolization."
- In collaboration with Paert A.L. and Boussauw L.
59. Lauweryns J. : - 11th Aspen Conference on research in emphysema, June 12-15, 1963, ASPEN (Colorado) - U.S.A.
- "Neonatal hyaline membrane disease : light and electron microscopic studies."
- By invitation of the Chronic Respiratory Disease Control Program of the United States Public Health Service.

60. Lauweryns J. : - University of Liège, Grand Lecture at the Viterbo of Liège.
 (Lecture in Liège of 1971, March 15, 1971).
 - Current View and Research concerning the pulmonary lymphatics.
61. Lauweryns J. : - Second International Congress of Lymphology, Miami, Florida,
 March 15-20, 1963.
 - "Macroscopic and histologic anatomy of pulmonary lymphatics."
 - In collaboration with Boussauw L.
62. Lauweryns J. : - Second International Congress of Lymphology, Miami, Florida,
 March 15-20, 1963.
 - "Pulmonary lymphatics in some diseases."
 - In collaboration with Boussauw L., Bourgeois N. and Claessens G.
63. Lauweryns J. : - 53^e Réunion de l'Association des Anatomistes, TOURS, April, 1961.
 - "Etude optique et ultrastructurale de l'innervation de papille
 gustative."
 - In collaboration with Vanherle G. and Fauskens J.
64. Lauweryns J. : - 53^e Réunion de l'Association des Anatomistes, TOURS, April, 1961.
 - "Les phospholipides pulmonaires et leur relation avec le facteur
 anti-atélectasique : étude histochoimique et dynamique."
 - In collaboration with Boussauw L.
65. Lauweryns J. : - Fourth European Regional Conference on Electron Microscopy,
 September 1-7, 1968, Rome (Italy).
 - "An electron microscopical study of neonatal hyaline membrane
 disease."
 - In collaboration with Bourgeois N. and Boussauw L.
66. Lauweryns J. : - 14th Annual Meeting of the Paediatric Pathology Society, 25-26
 October, 1968, ROTTERDAM (The Netherlands).
 - "Pulmonary ultrastructure in neonatal hyaline membrane disease."
 - In collaboration with Bourgeois N.
67. Lauweryns J. : - 54^e Congrès de l'Association des Anatomistes, March 30-April 3,
 1969, SOFIA (Bulgaria).
 - "Reconstruction graphique des valvules lymphatiques pulmonaire."
 - In collaboration with Boussauw L.
68. Lauweryns J. : - 12th Aspen Emphysema Conference, June 11 - June 14 1969, ASPEN,
 (Colorado) - U.S.A.
 - "The lymphatics of the lung : macroscopical and microscopical
 studies."
 - In collaboration with Burt A. and Boussauw L.

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70. Lauweryns J. : - "Neonatal Disease" - Department of Pathology, Middelheim Hospital, McGill University, June 17, 1969, Montreal, Canada.
- "The idiopathic respiratory distress syndrome of the newborn : light and electron microscopic studies."
71. (81) Lauweryns J. : - 24th session of the "Geneeskundige Dagen van Antwerpen", 16-22 September 1969, ANTWERP (Belgium).
- "Hyaline membranziekte van de pasgeborene" versus "idiopathic respiratory distress syndrome."
 - In collaboration with Bourgeois N.
72. (82) Lauweryns J. : - Boerhaave cursussen voor voortgezet medisch onderwijs, Rijksuniversiteit te Leiden, 27-29 November 1969, in LEXDEN (The Netherlands). General theme : "Physiology and pathology in the perinatal period."
- "Pathologische anatomie van de long in de perinatale periode."
73. (83) Lauweryns J. : - 2nd European Congress of Perinatal Medicine, 8-10 April 1970, LONDON (Great-Britain).
- "The unit lobule : a revised concept of the neonatal lung."
 - In collaboration with Rosen R.C.
74. (84) Lauweryns J. : - 7^e Congrès International de Microscopie Electronique, 30 August - 5 September 1970, GRENOBLE (France).
- "The freeze-etch ultrastructure of the interalveolar septum of the lung."
 - In collaboration with Gombeer-Desreucht M.
75. (85) Lauweryns J. : - 55^e Congrès de l'Association des Anatomistes, 22-26 March 1970, NANCY (France).
- "L'ultrastructure de la cloison interalvéolaire de la souris après cryodécapage."
 - In collaboration with Gombeer-Desreucht M.
76. (86) Lauweryns J. : - 55^e Congrès de l'Association des Anatomistes, 22-26 March 1970, NANCY (FRANCE).
- "L'ultrastructure de l'épithélium bronchique et bronchiolaire de la souris."
 - In collaboration with Cokelaere M. and Bousseaux L.
77. (87) Lauweryns J. : - 8th International Congress of the "International Academy of Pathology, 12-19 May 1970, MEXICO CITY (Mexico).
- "Neonatal hyaline membrane disease : light and electron microscopic studies."

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78. (91) Lauweryns J. : - "Morphological and morphological studies of the human lung in the neonatal period", in *Journal of Pathology and Bacteriology*, 1970, 134, 1-12 (London).
79. (92) Lauweryns J. : - "Morphology of the 'Human Lung of Pathology'", *Pathology*, 1970, 13, 1-12 (London).
80. (93) Lauweryns J. : - "Morphology of the 'Human Lung of Pathology'", *Pathology*, 1970, 13, 1-12 (London).
81. (94) Lauweryns J. : - Vergadering te LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research" van het INGO, met als thema "De long van de pasgeborene", op 13 April 1970.
82. (95) Lauweryns J. : - Vergadering in LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research" van het INGO, met als thema "De long van de pasgeborene", op 13 April 1970.
83. (96) Lauweryns J. : - Vergadering te LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research" van het INGO, met als thema "De long van de pasgeborene", op 13 April 1970.
84. (97) Lauweryns J. : - Vergadering te LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research" van het INGO, met als thema "De long van de pasgeborene" op 13 April 1970.
85. (98) Lauweryns J. : - Vergadering te LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research", van het INGO, met als thema "De long van de pasgeborene" op 13 April 1970.
86. (99) Lauweryns J. : - Vergadering te LIEVEN van de contactgroep aangaande "Pediatriesch Pathologisch Research" van het INGO, met als thema : "De long van de pasgeborene", op 13 April 1970.

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87. (100) Lauweryns J. : - "De kinder- en jeugdarts" van het "Pediatrisch Pathologisch Instituut" van het I.R.I., en als thema "De long van de pasgeborene", op 18 April 1970.
- Anatomopathologische studie van de neonatale hypoxische longaanvalten.
88. (101) Lauweryns J. : - Vergadering van de Nederlandse Vereniging voor Kinder- en jeugdartsen.
- "Oxygen pollution : its effect on the ecology of lung cells."
- In collaboration with Focan R.C.
89. (102) Lauweryns J. : - Joint Meeting of the "Pathological Societies of Ireland and England", in SHEFFIELD, (England), 10 July 1970.
- "Clinicopathological Aspects of Oxygen Toxicity of the Newborn Human and Animal (Bronchopulmonary Dysplasia).
- In collaboration with Rosan R.C.
90. (103) Lauweryns J. : - Symposium on "The lymphatic system of the heart and lungs" at the Third International Congress of Lymphology, August 27 - September 1, 1970, in BRUSSELS (Belgium).
- "The juxta-alveolar lymphatics of the lung."
91. (104) Lauweryns J. : - Symposium on "The lymphatic system of the heart and lungs" at the Third International Congress of Lymphology on August 27 - September 1, 1970 in BRUSSELS (Belgium).
- "The architecture of the pulmonary lymphatic valves."
- In collaboration with Boussauw L.
92. (105) Lauweryns J. : - Belgische Vereniging voor Pathologische Ontleedkunde.
- "Ultrastructure of the bronchiolar mucosa of the human premature infant."
- In collaboration with R.C. ROSAN.
93. (106) Lauweryns J. : - "Guest Lecture" at the "Department of Pathology" University of CRONINGEN, The Netherlands.
- "De ultrastructuur van de long van de menselijke pasgeborene."
94. (107) Lauweryns J. : - Koninklijk Vlaamse Academie voor Geneeskunde, 17 July in BRUSSELS (Belgium).
- "De ultrastructuur van het interalveolair septum en van het bronchiolair epitheel van de long na vrieestelling."
95. (108) Lauweryns J. : - "Guest Lecture" at the Department of Medicine, City of Hope Medical Center, May 19th, 1971, LOS ANGELES - U.S.A.
- "The fine structure of the lung lymphatics."

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10. (110) Lauweryns J. : - "Guest Lecture" at the Department of Medicine, Los Angeles County, University of Southern California Medical Center, May 12th, 1971.
- "Clinical and Experimental Aspects of Lung and Pulmonary Mortality."
27. (110) Lauweryns J. : - "Guest Lecture" at the Department of Medicine, Los Angeles County, University of Southern California Medical Center, May 12th, 1971.
- "Modern Concepts of Lung Structure, especially the blood and lymphatic microcirculation."
98. (111) Lauweryns J. : - "Guest Lecture" at the Department of Medicine, Los Angeles County, University of Southern California Medical Center, May 13th, 1971.
- Modern Concepts of Lung structure, especially the airways.
99. (112) Lauweryns J. : - "Guest Lecture" at the Department of Pathology, Hoffman Research Building, University of Southern California, Los Angeles.
- "Pathologic studies in neonatal hyaline membrane disease and its relationship to the idiopathic respiratory distress syndrome."
100. (113) Lauweryns J. : - "Guest Lecture" Department of Pediatrics, Cardinal Glennon Memorial Children's Hospital, St. Louis University, ST. LOUIS, (Missouri), April 26th 1971. (U.S.A.)
- "Hyaline Membrane Disease."
101. (114) Lauweryns J. : - "Guest Lecture" at the Department of Pathology, St. Louis University, ST. LOUIS, Missouri, - U.S.A. - April 26, 1971.
- "Pulmonary lymphatics."
102. (115) Lauweryns J. : - "Guest Lecture" Developmental Pathology Seminar, Department of Obstetrics and Gynecology, University of Chicago Lying-In Hospital, Chicago, ILL., U.S.A., April 29, 1971.
- "Lung development and perinatal pulmonary pathology."
103. (116) Lauweryns J. : - "Guest Lecture" at the Department of Pathology, University of Arizona Medical Center, May 3, 1971.
- "The Lung Lymphatics in health and disease."
104. (117) Lauweryns J. : - Amelia Block Memorial Lecturer 1971.
- Departments of Pathology and Medicine, Mount Zion Hospital and Medical Center, SAN FRANCISCO, California, U.S.A., May 6, 1971.
 - "The lung lymphatics : macroscopic and microscopic studies."

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105. (110) Lauweryns J. : - "The fine structure of the cell in the early stages of the disease" (by invitation).
106. (111) Lauweryns J. : - "The 1971 Annual Meeting of the American Thoracic Society Meeting, LOS ANGELES, California, U.S.A., May 17-19, 1971".
107. (120) Lauweryns J. : - "Guest Lecture" at the Anatomisches Institut der Universität Bern, Switzerland, January 10th, 1972.
108. (121) Lauweryns J. : - "Clinique de Gynécologie et d'Obstétrique de l'Université Libre de Bruxelles - Belgique, 9 February 1972".
109. (122) Lauweryns J. : - "Jaarvergadering van de Belgische Vereniging voor Electronenmicroscopie, 22 March 1972 in LEEUVEN (Belgium)".
110. (123) Lauweryns J. : - "Jaarvergadering van de Belgische Vereniging voor Electronenmicroscopie, 22 March 1972 in LEEUVEN (Belgium)".
111. (124) Lauweryns J. : - "57° Congrès de l'Association des Anatomistes et 22° Réunion de la 'Sociedad de Anatomía Portuguesa', 25-30 March 1972, in LISBONNE (Portugal)".
112. (125) Lauweryns J. : - "57° Congrès de l'Association des Anatomistes et 22° Réunion de la 'Sociedad de Anatomía Portuguesa', 25-30 March 1972, in LISBONNE (Portugal)".

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113. (126) Lauweryns J. : - Faculteit Medica 1971 de Pathologische Anatomie van de Longen in de Universiteitskliniek voor Kinderziekten, 1971, Antwerpen, (The Netherlands), 10 April 1971.
 - "Pathologische Anatomie van de Pyelische Nierziekten."
114. (127) Lauweryns J. : - 7th Congress of the European Society for Experimental Surgery, 11-14 April 1972, AMSTERDAM (The Netherlands).
 - "Morphological alterations after simultaneous bilateral lung homotransplantation (OLT) under various conditions."
 - In collaboration with Ch.R.H. Vildeveur and Ch. Jorvatier.
115. (128) Lauweryns J. : - Vergadering van de Belgische Vereniging voor Pathologische Anatomie, gewijd aan "De niet-tumorale pathologie van de long." BRUSSELS (Belgium), 10 June 1972.
 - "Pulmonary pathology of the newborn."
116. (129) Lauweryns J. : - "Guest Lecture" Department of Pathology, Nippon University, Tokyo - Japan, August 18th. 1972.
 - "Current ideas on the structure of neonatal and adult lung".
117. (130) Lauweryns J. : - "4th International Congress for Histochemistry and Cytochemistry", Kyoto, Japan, August 21-26, 1972.
 - "The ultrastructure of the lung alveoli using various fixatives and/or Freeze-etching".
 - In collaboration with Mrs. M. Gombeer-Desmecht.
118. (131) Lauweryns J. : - "International Conference on Pulmonary Reactions to Organic Materials" New York, November 8-11, 1972, organized by "The New York Academy of Sciences", Chairman Kaye H. Kilburn and Irving J. Selikoff.
 - "The lung lymphatics".
 - In collaboration with J. Baert.
119. (132) Lauweryns J. : - "4th International Congress of Lymphology" Tucson, Arizona, March 6-10, 1973.
 - The rôle of the lung lymphatics in the transport of intratracheally instilled particles.
 - In collaboration with J. Baert.
120. (133) Lauweryns J. : - Vergadering van de "Koninklijke Vlaamse Academie voor Geneeskunde van België", 24 february 1973.
 - "De lymfatische microcirculatie van de long".

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121. (134) Lauweryns J. : - Colloque International d'Histochemie, Tours (France),
1 - 4 July 1973.
- "Corpuscles Neuro-Epithéliaux de l'épithélium respiratoire des mammifères ; étude histochemique et ultrastructurale.
 - In collaboration with M. Cokelaere and P. Theunynck.
122. (135) Lauweryns J. : - International Symposium on Microcirculation, organized
by "The European Society of Cardiology", Lucerne
(Switzerland) 16-18 April 1973.
- Morphological studies of the blood and lymphatic microcirculation of the Lung.

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10-36471 } 2, 20-10-101 } 121.

- Born : **REDACTED**

- Location :

Scientific-Latin Unanities, St. Herbert College, Torhout

REDACTED

Incense.

University of Twente - Faculty of Sciences

REDACTED: "Licentiaat Biologische Wetenschappen" ("Master in Biological Sciences") (zoology) each year cum laude.

University of Leuven - Faculty of Medicine

1966-67 : Research Fellow "Vlaamse Leergangen" at the experimental laboratory.

1967 - till now : full-time university research assistant (University of Leuven)
at the experimental laboratory of cardiopulmonary and genital
pathology.

- Scientific lectures :

see lectures of Lammeryns, items: 39 ; 42 ; 47 ; 56 ; 58 ; 61 ; 62 ; 64 ; 65 ;
67 ; 68 ; 76 ; 84 ; 85 ; 91 ; 109 ; 112.

- Scientific publications :

see publications of Lauweryns, items : (49) ; (56) ; (57) ; (58) ; (60) ; (61) ;
(62) ; (66) ; (69) ; (70) ; (72) ; (73) ;
(74) ; (80) ; (82) ; (85) ; (86) ; (94) ;
(104) ; (114) ; (121) ; (124) ; (125) ; (131).

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FRS Acad. Valque (1967 - 1971), card. 1971-72.

- Form :

REDACTED

- Education :

Græco-Latin Humanities - magna cum laude.

University of Leuven - Faculty of Sciences

R (July) Licentiate Biologische Wetenschappen ("Master of Biological Sciences") (zoology) - cum laude.

University of Leuven - Faculty of Medicine

Since September **R** free collaborator and since January 1972, full-time university research assistant at the experimental laboratory of cardio-pulmonary and genital pathology.

Monique DESMET is a bright and hard working assistant who has gained precious experience in ultrastructure and in freeze-etching techniques.

- Scientific lectures :

see lectures of Lauweryns, items : 74 ; 75 ; 110.

- Scientific publications :

see publications of Lauweryns, items : (93) ; (97) ; (100) ; (120) ; (122).

1003539719

#908 - LEEDS

1003539720

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

May 30, 1973

Grant Application No. 908

TO: The Committee comprising Drs. Loosli, Sommers and Wyatt

SUBJECT: Sanford E. Leeds, M.D., Mt. Zion Med. Ctr., San Francisco
New Application No. 908
"Role of the pulmonary lymphatics in the absorption of inhaled particles and gases"

History

This proposal was case #145 and formal application was encouraged.

Application No. 908 requests \$56,485., plus two additional years.

Documents Submitted (attached)

1. Letter dated February 16, 1973.
2. Application dated 2-15-73.
3. Reprints of citations #1, 2, 4, 5 and 6 listed on page 3 of the application

FWN:gh
Encl.

FWN
F.W. N.

1003539721

Mount Zion Hospital and Medical Center



1600 Divisadero Street, San Francisco/Telephone (415) 567-6600
Mailing Address: Post Office Box 7921, San Francisco, California 94120

AIR MAIL

February 16, 1973

Executive Vice President
The Council For Tobacco Research-U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Re: Application for Research Grant
(Your case No. 145)

Dear Sir,

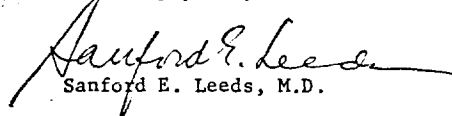
After correspondence with Dr. Nordsiek and Dr. Hockett, I am sending herewith an application for a research grant from the Council for Tobacco Research.

My colleagues and I believe it is an excellent project and will yield basic knowledge of the absorption of particles and gases. This knowledge will have useful applications in the field of air pollution.

A similar project was accepted by the Tuberculosis and Respiratory Disease Association of California after thorough screening and a site visit by Dr. Walter S. Tyler from the University of California at Davis. The project was also "favorably recommended" by the Environmental Sciences Advisory Committee. Both the above institutions were unable to fund the project.

I hope the application will receive favorable consideration.

Sincerely yours,


Sanford E. Leeds, M.D.

Encls.

Beneficiary Agency of the United Crusade and the Jewish Welfare Federation

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Drs. Loosli
Sommers
Wyatt

Physiology of Respiratory System

#908

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

FEB 21 1973

Application for Research Grant
(Use extra pages as needed)

Date:

2-15-73

1. Principal Investigator (give title and degrees):

Sanford E. Leeds, M.D.

2. Institution & address:

Mount Zion Hospital and Medical Center
1600 Divisadero Street
San Francisco, California 94115

3. Department(s) where research will be done or collaboration provided:

Experimental Surgery Laboratory

4. Short title of study:

Role of the pulmonary lymphatics in the absorption of inhaled particles
and gases

5. Proposed starting date: July 1, 1973

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

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7. Brief description of specific research aims

In order to understand the impact of air pollution on the human organism it is necessary to understand the route of absorption and the physiologic and pathologic effects of inhalation of particles and gases. An important and little understood pathway in the body for absorption of air pollutants is the extensive network of lymphatics in the lungs. The purpose of the experiments to be outlined is to study the absorption of non-toxic and toxic inhaled materials by the lungs with emphasis on the role of the lymphatic channels.

In general, our objective is to study the role of the pulmonary lymphatics in the absorption of inhaled particles and gases. The specific objectives are three-fold:

(1) To measure the amount of radioactive particles and gases in pulmonary lymph. This will quantitatively demonstrate the absorption of particles and gases by the pulmonary lymphatics.

(2) To determine the number and type of cells in right duct (RD), i.e., pulmonary lymph, and thoracic duct (TD) lymph after inhalation of particles and gases (irritant and non-irritant).

(3) To measure the flow and composition (cellular and chemical) of the hilar lymphatics. Lymph from these lymphatics is purely pulmonary without admixture of pericardial, cardiac, or other sources of lymph.

8. Brief statement of working hypothesis

The anatomic pathway from alveoli to lymphatics has been summarized by Yoffey and Courtice (1). Physiologic studies, such as those of Chinard (2), Schultz (3), and others, have demonstrated that absorption of various test substances into pulmonary capillaries occurs during the respiratory cycle when particle size and other factors are favorable.

The role of lymphatics in the absorption of RISA from the alveoli was studied by Meyer et al (4) who found that absorption of RISA by pulmonary capillaries was greater than that by the lymphatics. Since this is the only publication dealing specifically with pulmonary lymphatics, further studies seem desirable.

We, in this laboratory, have developed techniques for collecting pulmonary and systemic lymph which will permit quantitation of the uptake of absorbed particles (5,6). We also have initiated studies of the cell content of lymph from the right duct and thoracic duct of dogs (7). The proposed study will have practical application for control of air pollution by clarifying our understanding of basic mechanisms involved in the absorption and fate of inhaled particles and gases.

9. Details of experimental design and procedures

(1) Measurement of lymph flow and composition* of lymph from the RD and TD and blood serum after

- (a) endobronchial instillation of radioactive iodinated serum albumin (RISA);
- (b) inhalation of nebulized radio-aerosols in which the size and

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concentration of the aerosols particles is known. It is planned to use the following isotopes and labeled microspheres: (i) Small microspheres (0.25-3.0 μ) labeled with I^{125} ; (ii) Serum albumin tagged with I^{131} or I^{125} ; (iii) Xenon gas; (iv) Cl^{14} ; (v) Mono-dispersed fluorocarbon resin particles tagged with ^{18}F .

- (2) Measurement of lymph flow and composition* of lymph from RD and TD after inhalation of

- (a) irritant gases,
- (b) irritant dusts.

- (3) Radioautographs of lung to show fate of radioactive particles and gases.

*Analysis of composition of lymph includes the following: Chemical composition - electrolytes, protein, oxygen, enzymes, etc., and cellular composition - total cell count and differential count. Radioactivity is measured when indicated.

All experiments employ dogs. Endobronchial instillation of RISA is by bronchoscopy and the introduction of a 16 gauge polythene catheter (Cobe Catheter Co.) into each main stem bronchus. Two ml RISA are injected directly into the right and left main stem bronchus followed by 2 ml saline to wash out the catheter. A cuffed endobronchial tube is inserted immediately on removing the bronchoscope in order to avoid loss of RISA. In other experiments radio-aerosols are administered. The size and concentration of the aerosol particles are regulated. For this purpose a respirator is used with nebulizer, filter and exhalation manifold. When irritating particles and gases are administered the animal is placed in a fume hood.

The method of cannulation of the pulmonary lymphatics (RD) has been described by Leeds et al (5). Briefly, the RD and TD are cannulated in the neck, the chest is not opened. Evans blue dye (T-1824) is instilled into the right lung in order to aid in visualizing the leash of fine lymphatics which makes up the RD. A segment of the right external jugular and subclavian veins into which the pulmonary lymphatics drain is isolated by dissection and ligation, taking care to preserve the lymphatics. The segment of the external jugular vein is then cannulated and the entire pulmonary lymph drainage, except for a portion from the left upper lobe, is collected. This is an improvement over the method of Warren and Drinker (8) published in 1942, in which one small lymphatic was cannulated and only a small portion of the pulmonary flow obtained. The TD is cannulated in the neck near its junction with the venous system. Five hourly samples of lymph from the RD and TD are usually collected for measurement of rate of flow and for analysis.

Scintillation counts are done by the Department of Nuclear Medicine under the direction of Dr. Kenneth R. McCormack to determine radioactivity in 1 cc samples of lymph. The remaining lymph samples are used for chemical tests and cellular determinations. Blood is drawn at hourly intervals and radioactivity of 1 cc aliquots of serum are measured.

The method of counting cells has been described by Leeds et al (7). The cell counts and differential counts of morphologic types are performed in the Immunology Research Laboratory under the direction of Dr. Ernest H. Rosenbaum,

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The technique of cannulation of hilar lymphatic is similar to that employed for cannulation of a cardiac lymphatic (Leeds et al (9)). Evans blue dye (T-1824) is injected into the lung parenchyma distally and the hilar lymphatics are thus visualized. Using a Zeiss operating microscope for magnification, a fine polythene tube is inserted into an isolated lymphatic and tied in place with 6-0 Deknatel silk. The polythene catheter is washed with dilute heparin solution prior to insertion. Lymph samples are collected hourly to measure rate of flow and to study cellular and chemical characteristics. Following instillation or inhalation of radioactive materials, radioactivity of the lymph is measured by the Nuclear Medicine Laboratory.

Noxious gases and irritant particles are administered by aerosol nebulizer in a fume hood into which the anesthetized dog is placed. Radioautographs are done by standard methods when indicated.

10. Space and facilities available

These include a modern experimental surgery laboratory, opened in 1963, with four operating tables each with piped-in suction, air and oxygen, special lights and Bird respirator. Large preparation and work rooms adjoin. In addition, there is an individual room for the use of the investigators and technician personnel which contains benches, desk, record files, book shelves, storage cupboards and drawers for special equipment. Major items of permanent equipment include an x-ray room complete with equipment for roentgenography and fluoroscopy, pumps and oxygenators, electrocardiograph, electronics for medicine, and other recording devices and manometers and a Zeiss operating microscope. The latter is equipped with camera and Strobe light for rapid exposures.

In addition, the facilities of the Division of Nuclear Medicine are available for determinations of radioactivity in blood and lymph and for scintillation scans and radioautographs of the lung. The facilities of the Immunology Research Laboratory are available for cell counts of lymph, hematologic and immunologic (e.g., electrophoresis) studies. The facilities of the Pulmonary Laboratory are available for administration of aerosol particles and gases. The personnel of these three sections collaborate closely with the principal investigator and his associates in a team effort. All the above facilities are under one roof - the Mount Zion Hospital and Medical Center.

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Biographical sketches of investigators and other professional personnel (append)

Publications

1. Leeds SE, Uhley HN, Sampson JJ, Friedman M: A new method for measurement of lymph flow from the right duct in the dog. Am J Surg 98:211, 1959.
2. Leeds SE, Uhley HN, Sampson JJ, Friedman M: Significances of changes in the pulmonary lymph flow in acute and chronic experimental pulmonary edema. Am J Surg 114:254, 1967.
3. Ortega P, Uhley HN, Leeds SE, Friedman M, Sampson JJ: Serial electron and light microscopic studies in the dog lung in chronic experimental pulmonary edema. Am J Path 60:57, 1970.
4. Leeds SE, Reich SB, Uhley HN, Sampson JJ, Friedman M: The pulmonary lymph flow after irradiation of the lungs of dogs. Chest 59:203, 1971.
5. Leeds SE, Uhley HN, Basch CM, Rosenbaum EH, Yoffey JM: Comparative study of lymph and lymphocytes of the thoracic and right lymphatics ducts. I. Normal dogs. Lymphology 4:53, 1971.
6. Uhley HN, Leeds SE, Sung AM: The subendocardial lymphatics of the canine heart - A possible role of the lymphatics in the genesis of conduction disturbances and arrhythmias. Am J Cardiol 29:367, 1972.
7. Mann PEG, Cohen AB, Finley TN, Ladman AJ: Alveolar macrophages. Structural and functional differences between nonsmokers and smokers of marijuana and tobacco. Lab Invest 25:111-120, 1971.

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14. First year budget:

A. Salaries (give names or state "to be recruited")		% time	Amount
Professional (give % time of investigator(s) even if no salary requested)			
Sanford E. Leeds, M.D.	Principal Investigator	30	
Philip E.G. Mann, M.D.	Co-Investigator	20	
Herman N. Uhley, M.D.		5	
Theodore N. Finley, M.D.		5	
Kenneth R. McCormack, M.D.		5	
Ernest H. Rosenbaum, M.D.		5	

Technical

Vaida Hoffman, R.N.,	Technician II	100	
To be recruited	Asst. Tech. II	100	
Anna S. Vikart	Secretary	16½	
To be recruited	Animal Diener	20	
Fringe Benefits			

Sub-Total for A

B. Consumable supplies (by major categories)

Dogs at \$25.00 each	\$2500
Surgical supplies, endotracheal tubes, plastic tubing, etc.	500
Radioactive isotopes	1000
Oxygen, miscellaneous gases	200

Sub-Total for B 4,200

C. Other expenses (itemize)

Publication	300	
Photography, artist	75	
Laboratory tests, nuclear medicine		
laboratory costs	500	
Travel to Air Pollution Symposia	<u>500</u>	
	Sub-Total for C	<u>1,375</u>

Running Total of A + B + C 48,248

D. Permanent equipment (itemize)

Ultrasonic nebulizer	350
Respirator with nebulizer, filter and exhalation manifold	650

Sub-Total for D 1,000

E. Indirect costs (15% of A+B+C)

E 7,237

Total request 56,485

15. Estimated future requirements.

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	4,200	1,375	-	7,589	58,184
Year 3		4,200	1,375	-	7,960	61,031

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5.

16. Other sources of financial support-

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Relation of Lymphatics to Cardiopulmonary Function	Department of Health, Education and Welfare HL 3180-15 (Suppl.)	\$6,795	Jan. 1-Mar.31, 1973 After March 31, 1973 we have no funds unless Congress appropriates funds for new 5-year grant. This appears to be doubtful.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Relation of Lymphatics to Cardiopulmonary Function	Department of Health, Education and Welfare HL 3180-15	\$276,759 (First year \$51,044)	Dec. 1, 1973 - Nov.30, 1978 (Dec. 1, 1973 - Nov.30, 1974)

N.B. This grant has been awarded and is pending action by Congress and the President to appropriate the necessary funds.

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Sanford E. Leeds, M.D.Signature *Sanford E. Leeds* Date 2/14/73

Telephone _____

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Area Code _____ Number _____ Extension _____

Checks payable to

Mount Zion Hospital & Medical Center

Mailing address for checks

Mr. George A. Thompson, Controller
Mount Zion Hospital & Medical Center
P. O. Box 7921, San Francisco, Ca. 94120

Responsible officer of institution

Typed Name Jay Okun Yedwab,Title Executive DirectorSignature *Jay Okun Yedwab* Date 2/15/73

Telephone 415 567-6600 201
Area Code _____ Number _____ Extension _____

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CURRICULUM VITAE

SANFORD E. LEEDS, M.D.

Date and place of birth :

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Education:

R

University of California, A.B.

University of California Medical School, M.D.

1935 - 1936 Intern, San Francisco Hospital, University of California Service

Positions held:

1936 - 1938 Research Associate, Vanderbilt University Medical School,
Nashville, Tennessee, with Dr. Alfred Blalock.

1938 - 1941 Assistant Resident and Resident in Surgery, University of
California Hospital and UC Service, San Francisco Hospital.

1941 - 1949 Active Duty, U.S. Army, 1st Lt. to Lt. Col., New Guinea and
Luzon Campaigns. Col. M.C., USAR, retired in 1968 after
30 years service in active reserve.

1941 - 1952 Clinical Instructor of Surgery.

1952 - Assistant Clinical Professor of Surgery, University of California
Medical School.

1952 - Associate Chief of Surgery, In Charge of Cardiac Surgery (1952-63),
Mount Zion Hospital and Medical Center.

Present: Assistant Clinical Professor of Surgery, University of California
Medical School, San Francisco;
Associate Chief of Surgery (1952 - present), and
Director, Experimental Surgery Laboratory (1969 - present),
Mount Zion Hospital and Medical Center, San Francisco.

Specialty Certification:

American Board of Surgery - 1942

The Board of Thoracic Surgery - 1952

Professional Societies:

REDACTED

Curriculum Vitae - Sanford E. Leeds, M.D., continued

REDACTED

Author of various papers, to date, January 1973 - 75 papers.

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CURRICULUM VITAEHERMAN N. UHLEY, M.D.

REDACTED

Date and place of birth:Undergraduate and Graduate Education:

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University of Wisconsin

University of Wisconsin School of Medicine

Postgraduate Training:

Internship, Michael Reese Hospital, Chicago, Illinois	1951-52
Research Fellow, Michael Reese Hospital, Chicago, Ill.	1952-53
Resident, Beth Israel Hospital, Boston, Massachusetts	1953-55
Teaching Fellow, Harvard Medical School, Boston, Mass.	1953-55
Research Fellow, Harold Brunn Institute, Mount Zion Hospital and Medical Center, San Francisco, California	1955-56

Positions held:

Assoc. Clinical Professor of Medicine, University of California School of Medicine, San Francisco, California.

Asst. Chief of Medicine, Mount Zion Hospital and Medical Center, San Francisco.

Attending Physician, Medicine, V. A. Hospital, San Francisco.

Assoc. Chief of Medicine, In Charge of Department of Electrocardiography, Mount Zion Hospital and Medical Center, San Francisco.

Present: Assoc. Chief of Medicine, and Director, Department of Electrocardiography and Coronary Care Unit, Mount Zion Hospital and Medical Center, San Francisco; Assoc. Clin. Prof., Medicine, UCSF School of Medicine, San Francisco.

Society Membership:

REDACTED

Specialty Certification:

American Board of Internal Medicine, 1961

Publications:

Book: Vector Electrocardiography, Lippincott, Philadelphia, Pa., 1962.

Papers: To date, January 1973 - 62 articles.

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HERMAN N. UHLEY, M.D.

LIST OF PUBLICATIONS.

BOOK:

VECTOR ELECTROCARDIOGRAPHY, Lippincott, Philadelphia, Pa., 1962.

PAPERS:

1. Uhley, H.N.: A model for automatic determination of the relationship between the cardiac vector and the three standard limb leads. J. Appl. Physiol. 6:260-262, 1953.
2. Uhley, H.N., M. Friedman, C. Ayello: The atherosclerotic response of "aggressive" and "passive" groups of chickens to cholesterol enriched diet. Proc. Soc. Exper. Biol. Med. 96:244-246, 1957.
3. Friedman, M., H.N. Uhley: Experimental stress, blood lipids and atherosclerosis. In: HORMONES AND ATHEROSCLEROSIS, Chapter 16, pp.205-211, Academic Press, Inc. New York, 1959.
4. DeLong, E., H.N. Uhley, M. Friedman: Change in blood clotting time of rats exposed to a particular forms of stress. Am. J. Physiol. 196:429-430, 1959.
5. Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman: A new method for measurement of lymph flow from the right duct in the dog. Am. J. Surg. 98:211-216, 1959.
6. Uhley, H.N., L.M. Rivkin: Visualization of the left branch of the human atrioventricular bundle. Circulation 20:419-421, 1959.
7. Friedman, M., H.N. Uhley: Role of the adrenal in hastening blood coagulation after exposure to stress. Am. J. Physiol. 197:205-206, 1959.
8. Uhley, H.N., M. Friedman: Blood lipids, clotting and coronary atherosclerosis in rats exposed to a particular form of stress. Am. J. Physiol. 197:396-398, 1959.
9. Uhley, H.N., S.E. Leeds, J.J. Sampson, M. Friedman: Right duct lymph flow in dogs measured by a new method. Dis. Chest 37:532-534, 1960.
10. Uhley, H.N., L.M. Rivkin: Peripheral distribution of the canine A-V conduction system. Observations on the gross morphology. Am. J. Cardiol. 5:688-691, 1960.
11. Uhley, H.N., S.B. Reich, L.M. Rivkin: Radioautography of the conduction system of the dog's heart with I¹³¹. Am. J. Physiol. 198:859-860, 1960.

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12. Rivkin LM, H.N. Uhley: Electrocardiographic alterations induced by incisions in the distal conduction system. Surg Forum 11:202-204, 1960.
13. Uhley HN: Study of the transmembrane action potential, electrogram, electrocardiogram and vectorcardiogram of rats with left ventricular hypertrophy. Am. J. Cardiol. 7:211-217, 1961.
14. Uhley H.N., S.E. Leeda, J.J. Sampson, M. Friedman: Some observations on the role of the lymphatics in experimental acute pulmonary edema. Circ. Res. 9:688-693, 1961.
15. Uhley, H.N., L.M. Rivkin: Electrocardiographic patterns following interruption of main and peripheral branches of the canine right bundle of His. Am. J. Cardiol. 7:810-816, 1961.
16. Uhley, H.N., S.E. Leeds, J.J. Sampson, M. Friedman: Role of the pulmonary lymphatics in chronic pulmonary edema. Circ. Res. 11:966-970, 1962.
17. Burton S.D., H.N. Uhley, T. Ishida: Technique for recording multiple avian embryonic electrocardiograms. J. Appl. Physiol. 18:650-651, 1963.
18. Uhley, H.N., S.E. Leeda, J.J. Sampson, M. Friedman: A technic for collection of right duct lymph flow in unanesthetized dogs. Proc Soc Exp Biol Med 112:684-685, 1963.
19. Uhley, H.N., D.E. Bernstein: Termination of ventricular tachycardia by external countershock. California Med 98:281-282, 1963.
20. Uhley HN, L.M. Rivkin: Electrocardiographic patterns following interruption of the main peripheral branches of the canine left bundle of His. Am. J. Cardiol. 13:41-47, 1964.
21. Uhley, H.N., Lewis, A.E., G.R. Biskind: A simple device for checking the Coulter counter. Am. J. Clin. Path. 44:600-601, 1965 (reprinted from Techn Bull Registry Med Technol Vol.35, No.10,1955).
22. Uhley, H.N., L.M. Rivkin: Cardiac slowing induced by external coupled pacing. Bull New York Acad. Med., 2nd Series, 41:565-570, No.5,1965.
23. Rivkin, L.M., H.N. Uhley: Slowing of the heart rate with synchronized delayed single pulse pacing. Clin.Res. 13:122, 1965 (abstract).
24. Uhley, H.N., E. Bossi: Rapid mass electrocardiographic screening in college students. J. Am. Coll. Assn. 13:341-346, 1965.
25. Uhley, H.N., H.H. Rosenblum: Electrocardiographic limb leads: Suggestion for their logical display. Am Heart J 71:571-573, 1966.
26. Rivkin, L.M., H.N. Uhley: Effect on heart rate, aortic flow and left ventricular pressure induced by coupled pacing. Dis Chest 49:512-515, 1966.

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27. Sampson, J.J., H.N. Uhley, S.E. Leeds, M. Friedman: The relation of pulmonary lymph flow to pulmonary edema: The protective action of an expanded lymph drainage system. Proc. World Congress of Cardiology, New Delhi, India, 1966.
28. Uhley, H.N., S.E. Leeds, J.J. Sampson, N. Rudo, M. Friedman: The temporal sequence of lymph flow in the right lymphatic duct in experimental chronic pulmonary edema. Am. Heart J. 72:214-217, 1966.
29. Cohen, R.A., H.N. Uhley: Monitoring the blood pH in acute myocardial infarction. The role of acidosis in arrhythmia. JAMA 198:947-949, 1966.
30. Uhley, H.N., S.E. Leeds, J.J. Sampson, M. Friedman: Right duct lymph flow in experimental heart failure following acute elevation of left atrial pressure. Circ. Res. 20:306-310, 1967.
31. Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman: Significance of changes in the pulmonary lymph flow in acute and chronic experimental pulmonary edema. Am. J. Surg. 114:254-258, 1967.
32. Friedman, M., H.N. Uhley: Management of coronary heart disease. Postgrad. Med. 42:155-164, 1967.
33. Sampson, J.J., S.E. Leeds, H.N. Uhley, M. Friedman: The Lymphatic System and Pulmonary Disease. In: LYMPH AND THE LYMPHATIC SYSTEM, Charles C Thomas, 1968, Chapter IX, pp.200-212. (Proceedings of the Conference on Lymph and Lymphatic System, New Orleans, December, 1965).
34. Sampson, J.J., S.E. Leeds, H.N. Uhley, M. Friedman, P. Ortega: Studies on the lymph flow and changes in pulmonary structures as indices of circulatory changes in experimental pulmonary edema. Israel J. Med. Sci. 4:116, 1968. (Presented at Fourth Asian-Pacific Congress of Cardiology, Sept.1-7, 1968, Jerusalem and Tel Aviv, Israel.)
35. Uhley, H.N.: The use of 6-channel ECG recorders in improving the efficiency of an Electrocardiography department. J. Electrocardiography, 2:69-72, 1969.
36. Uhley, H.N., S.E. Leeds, J.J. Sampson, M. Friedman: The cardiac lymphatics in experimental chronic congestive failure. Proc. Soc. Exper. Biol. Med. 131:379-381, 1969.
37. Uhley, H.N.: A new simple model for the synthesis of the electrocardiogram. Circulation 40:173-178, 1969.
38. Ziporovich, S., H.N. Uhley, H.W. Paley: Coronary nodal rhythm following open heart surgery. Dis. Chest 55:219-223, 1969.

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39. Sampson, J.J., S.E. Leeds, H.N. Uhley, M. Friedman, P. Ortega: Studies of lymph flow and changes in pulmonary structures as indexes of circulatory changes in experimental pulmonary edema. *Israel J. Med. Sci.* 5:826-830, 1969.
40. Uhley, H.N.: Electrical monitoring of the acutely ill patients. *Geriatrics* 25:145-150, 1970.
41. Uhley, H.N.: A simple means of determining blood loss in urinary drainage. *J. Urology* 104:328-329, 1970.
42. Ortega, P., H.N. Uhley, S.E. Leeds, J.J. Sampson: Electron~~ic~~ and light microscopy studies of progressive pulmonary edema in experimental heart failure in the dog (with special reference to expansion of pulmonary lymph flow with congestive heart failure). In: *PROGRESS IN LYMPHOLOGY II*. Georg Thieme Verlag, Stuttgart, 1970, pp.15-20. 32-34. (Selected papers of the Second International Congress of Lymphology, Miami, USA, March 15-20, 1968).
43. Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman: Changes in the pulmonary lymph flow in acute and chronic experimental pulmonary edema. In: *PROGRESS IN LYMPHOLOGY II*. Georg Thieme Verlag, Stuttgart, 1970, pp.261-264. (Selected papers of the Second International Congress of Lymphology, Miami, USA, March 15-25, 1968).
44. Ortega, P., H.N. Uhley, S.E. Leeds, M. Friedman, J.J. Sampson: Serial electron and light microscopic studies on the dog lung in chronic experimental pulmonary edema. *J. Path.* 60:57-74, 1970.
45. Uhley, H.N.: Electrocardiographic telemetry from ambulances. A practical approach to mobile coronary units. *Am. Heart J.* 80:838-842, 1970.
46. Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman: The cardiac lymphatics after ligation of the coronary sinus. *Proc. Soc. Exper. Biol. Med.* 135:59-62, 1970.
47. Uhley, H.N., A.E. Brown, M. Friedman, H. Rosenblum, S.R. Sherman, F.F. Stucki, J.T. Wilson: Automatic surveillance of rate, rhythm and T wave contour: the concept of monitoring large number of patients. *Am. J. Cardiol.* 26:375-378, 1970.
48. Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman: Measurement of lymph flow of the heart. Abstract in Program of 3rd International Congress of Lymphology, Brussels, Aug.27-Sept. 1, 1970.

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49. Leeds, S.E., H.N. Uhley, E.H. Rosenbaum, C.M. Basch, J.M. Yoffey: Comparative studies of lymph and lymphocytes of the thoracic and right lymphatic ducts in normal dogs collected in five hourly periods. Abstract in Program of the 3rd Internat. Congress of Lymphology, Brussels, Aug.27-Sept 1, 1970.
50. Leeds, S.E., S.B. Reich, H.N. Uhley, J.J. Sampson, M. Friedman: The pulmonary lymph flow after irradiation of the lungs of dogs. Chest 59:203-207, 1971.
51. Leeds, S.E., S.B. Reich, H.N. Uhley, J.J. Sampson, M. Friedman: The pulmonary lymph flow after irradiation of the lungs of dogs. (Review of publication in Chest, abstract and reviewed by Dr. R.G.Fraser) Invest. Radiology 6:440, 1971.
52. Uhley, H.N.: Telemetry in mobile coronary care. Med Opinion & Review 7:52-54, 1971.
53. Uhley, H.N., S.D. Burton, A.E. Lewis, T. Ishida: Electrocardiographic monitoring of the embryonic duck heart Digitoxin assay. J. Biomed. Systems 2:10-13, 1971.
54. Leeds, S.E., H.N. Uhley: Measurement of lymph flow of the heart. Lymphology 4:31-34, 1971.
55. Leeds, S.E., H.N. Uhley, C.M. Basch, E.H. Rosenbaum, J.M. Yoffey: Comparative study of lymph and lymphocytes of the chronic and right lymphatic ducts. I. Normal dogs. Lymphology 4:53-57, 1971.
56. Uhley, H.N., S.E. Leeds, M.-A. Sung: The subendocardial lymphatics in the genesis of conduction disturbances and arrhythmias. Am J Cardiol 29:367-371, 1972.
57. Uhley, HN: Some controversy regarding the peripheral distribution of the conduction system. Am J Cardiol 1972 (accepted for publication).
58. Uhley HN: The quadrifascicular nature of the peripheral conduction system. In: Cardiac Arrhythmias. LS Dreifus and W Likoff, eds., 1972 (accepted for publication).
59. Hecht H et al: Conduction system nomenclature. Am. J Cardiol 1972, (accepted for publication).
60. Uhley HN: Automatic monitoring in the hospital. Hospital Practice 1972, (accepted for publication).
61. Uhley HN: Monitoring of the surgical patient. Contemporary Surgery 1972, (accepted for publication).
62. Uhley HN: Fascicular block (in preparation for Cardiac Clinics).

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CURRICULUM VITAETHEODORE N. FINLEY, M.D.Date and place of birth

REDACTED

Education and training :

University of Washington

B.S. (Chemistry)

Johns Hopkins Medical School

M.D.

Internship: San Francisco County Hospital

Residency : University of California Medical Center
and San Francisco County HospitalScholarships or Fellowships held:

Research Fellow, University of Buffalo, Dept. Physiology

1957-1958

Research Fellow, American Trudeau Society, University of California

1958-1961

Positions held:Clinical Instructor in Medicine, Cardiovascular Research Institute,
University of California Medical Center

1958-1961

Assistant Professor of Anesthesiology, Physiology and Biophysics,
and Director of Anesthesiology Research, University of Washington
School of Medicine

1961

Associate Professor, University of Washington School of Medicine

1964 (July)

Associate Professor of Medicine, University of New Mexico
School of Medicine

Sept. 1964-1968 (June)

Associate Chief of Medicine and Director, Pulmonary Laboratory,
Department of Medicine, Mount Zion Hospital & Medical Center

June 1968-present

Associate Clinical Professor, University of California Medical
Center, San Francisco

June 1968-present

Medical Director, Inhalation Therapy, Mount Zion Hospital
and Medical Center

1968-present

Research appointments: see aboveBoard Certification: Qualified for Internal MedicineProfessional and Scientific Memberships:

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REDACTED

BIBLIOGRAPHY

1. Finley TN, Lenfant C, Haab P, Pilper J, Rahn H: Venous admixture of pulmonary circulation of anesthetized dog. *J Appl Physiol* 15:419, 1960.
2. McIlroy MB, Butler J, Finley TN: Effects of chest compression on reflex ventilation drive and pulmonary function. *J Appl Physiol* 17:701, 1962.
3. Finley TN: The relative efficiency of gas transport in the lung in relation to the ventilation perfusion ratio. WADD Techn Report 60-1, 1960.
4. Swenson EW, Finley TN, Guzman SV: Unilateral hypoventilation during temporary ipsilateral pulmonary artery occlusion in man. *J Clin Invest* 40:828, 1961.
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6. Finley TN: The determination of uneven pulmonary blood flow from the arterial oxygen tension during nitrogen washout. *J Clin Invest* 40:1727, 1961.
7. Finley TN, Swenson EW, Comroe Jr, JH: The cause of arterial hypoxemia at rest in patients with "alveolo-capillary syndrome". *J Clin Invest* 41:618, 1962.
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9. Finley TN: Pulmonary surface activity and the problems of atelectasis, wetting, foaming and detergency in the lung. *Anesth Analges* 42:35, 1963.
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14. Morgan TE, Finley TN, Huber GL, Fialkow H: Alterations in pulmonary surface active lipids during exposure to increased oxygen tension. *J Clin Invest* 44:1737, 1965.

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Theodore N. Finley, Bibliography, continued

15. Morgan TE, Finley TN, Fialkow H: Comparison of the composition and surface activity of "alveolar" and whole lung lipids in the dog. *Biochem Biophys Acta* 106:403, 1965.
16. Huber GL, Edmunds LH, Finley TN: Acute effects of saline lavage on pulmonary mechanics and morphology. *Surg Forum* 17:113, 1965.
17. Finley TN, Swenson EW, Curran WS, Huberg GL, Ladman AJ: Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. *Ann Int Med* 66:651, 1967.
18. Ladman AJ, Pratt SA, Finley TN: Exfoliative cytology of the lung alveolus: Preliminary electron microscopic observations on cells obtained in vivo from human lungs. 25th Annual EMSA Meeting.
19. Ladman AJ, Finley TN, Brewer L, MacKay ME: A morphological and lipid analysis of the alveolar lining material in the dog. *J Lipid Res* 9:357, 1968.
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27. Finley TN, Ladman AJ: Low yield of pulmonary surfactant in cigarette smokers. *New Eng J Med* 286:223, 1972.

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CURRICULUM VITAEERNEST H. ROSENBAUM, M.D.**REDACTED**Date and place of birth:Education:

University of New Mexico

University of Colorado

Internship: San Francisco General Hospital

Residency : San Francisco General Hospital;

Mount Zion Hospital and Medical Center

Positions held:

U.S.A.F. Medical Corps.

1959 -

Hematology Fellow, Blood Research Laboratories,

New England Center Hospitals, Boston, Mass.

1959 - 1961

Massachusetts Institute of Technology - Courses in

Molecular Genetics, Radioisotopes, Immunology and

Biochemistry

1961 - 1962

Instructor of Clinical Medicine, University of
California School of Medicine, San Francisco, Ca.

1966 - present

Director, Medical Cancer Service, Mount Zion
Hospital and Medical Center

1968 - 1971

Associate Chief of Medicine, Chief, Clinical

1971 - present

Immunology, Department of Medicine, and Director,

Immunology Research Laboratory, Mount Zion Hospital

and Medical Center

Society Membership:**REDACTED**

1003539741

Ernest H. Rosenbaum, M.D.

BIBLIOGRAPHY

1. Kiossoglou K, Rosenbaum EH, Mitus J, Dameschek W: Multiple chromosomal aberrations in a patient with acute granulocytic leukemia with Down's syndrome and Twinning. *Blood* 24:134, 1964.
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3. Ross J, Rosenbaum EH: Paroxysmal nocturnal hemoglobinuria presenting as aplastic anemia in a child. *Am J Med* 37:130, 1964.
4. Sbarra A, Shirley W, Rathman J, Ouchi E, Rosenbaum EH: The role of the phagocyte in host-parasite interactions. *Cancer Res* 24:1958, 1964.
5. Sbarra AJ, Shirley W, Selvaraj RJ, McRipley RJ, Rosenbaum EH: The role of the phagocyte in host-parasite interaction. III. The phagocytic capabilities of leukocytes from myeloproliferative and other neoplastic disorders. *Cancer Res* 25:1199, 1965.
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7. Rosenbaum EH, Cohen RA, Gladstein HB: Vaccination of a patient receiving immunosuppressive therapy for lymphosarcoma. *JAMA* 198:737, 1966.
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9. Basch CM, Rosenbaum EH: Clinical correlation of immunoglobulin changes with therapy in multiple myeloma. *California Med* 110:1, 1969.
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I. Normal dogs. *Lymphology* 4:53, 1971.
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12. Rosenbaum EH, Basch CM, Fudenberg HH: Mixed cryoglobulinemia with cryoglobulinuria. In preparation.
13. Rosenbaum EH, Basch CM, Leeds SE, Uhley HN, Yoffey JM: Comparative study of lymph and lymphocytes of the thoracic and right lymphatic ducts.
II. Dogs in congestive heart failure. In preparation.
14. Rosenbaum EH, Miner R, Basch CM, Harper K: Host defense mechanisms. *Front. Radiation Ther. Onc.* 7:50-64, 1972.

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CURRICULUM VITAEPHILIP E. G. MANN, M.D.College

Georgetown University, Washington, D.C.
 American University of Beirut, Beirut, Lebanon

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Professional

American University of Beirut School of Medicine 1956-1961

Residency (Anesthesiology)

New England Deaconess Hospital, Boston, Mass. 1961-1962
 Mary Hitchcock Memorial Hospital, Hanover, N.H. 1962-1963
 (Dartmouth Affiliated Hospital)

Fellowship

San Francisco T.B. Association 1970-1971
 Fellow, Pulmonary Laboratory, Mount Zion Hospital
 and Medical Center, San Francisco, Ca. 1968-1970

Appointments

National Institutes of Health
 Division of Anesthesiology 1963-1968
 Assistant Director, Pulmonary Laboratory,
 Mount Zion Hospital and Medical Center 1971-1972
 Adjunct in Medicine, Mount Zion Hospital
 and Medical Center 1971-1972
 Anesthesiologist, St. Joseph Hospital,
 San Francisco, Ca. 1972-

Degrees

B.Sc. - American University of Beirut 1957
 M.D. - American University of Beirut 1961

Societies (Honorary)

Alpha Omega Alpha 1961

Professional Organizations

REDACTED

Certification

E.C.F.M.G. - Standard Certificate 220223 1961
 American College of Anesthesiologists -
 Fellow Certificate 2944 1964
 American Board of Anesthesiology (Part 1) 1972

Licensure

State of Pennsylvania - by examination 1962
 R
 State of California - by examination

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1. Tunner, W.S., Kiser, W.S., Mann, P.E.G.: Studies on the significance of decreased serum sodium levels following the clinical use of mannitol. J. Urol. 94:470-474, 1965
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3. Cooperman, L.H., Mann, P.E.G.: A simple method for direct arterial pressure measurement. Anesthesiology 27:93-94, 1966
4. Cooperman, L.H., Mann, P.E.G.: Anesthetic management of pheochromocytoma employing halothane and beta adrenergic blockade. A report of fourteen cases. Anesthesiology 28:575-582, 1967
5. Goodman, S.J., Mann, P.E.G.: Reticular and thalamic multiple unit activity during wakefulness, sleep and anesthesia. Exper. Neurol. 19:11-24, 1967
6. Mann, P.E.G., Boretos, J.: A low dead space feline anesthetic mask. Lab. Animal Care. 18:657-660, 1968
7. Mann, P.E.G., Cohen, A.B., Finley, T.N., Ladman, A.J.: Alveolar Macrophages: Structural and Functional Differences between Nonsmokers and Smokers of Marijuana and Tobacco. Lab. Invest. 25:111-120, 1971

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CURRICULUM VITAEKENNETH R. McCORMACK, M.D.Date and place of birth:REDACTED
REDACTEDEducation:

University of Idaho
 McGill University Medical School
 Internship: Good Samaritan Hospital,
 Portland, Oregon
 Residency: Oakland, Ca., Columbus, Ohio

R

REDACTED

Positions held:

Instructor in Radiology, Asst. Radiologist, University of California School of Medicine	1958-60
Assistant Professor of Radiology, Asst. Radiologist, University of California School of Medicine	1960-66
Associate Professor of Radiology, Assoc. Radiologist, University of California School of Medicine	1966
Associate Professor of Radiology, University of Kentucky	1966-67
Assoc. Director, Claire Zellerbach Saroni Tumor Institute, Mount Zion Hospital and Medical Center	1967-72
Assoc. Chief, Department of Laboratory and Pathology Medicine, and Director, Division of Nuclear Medicine, Mount Zion Hospital and Medical Center	1972-present
Associate Clin. Professor of Radiology, University of California School of Medicine	1967-present

Consultant:

U. S. Naval Hospital, Oak Knoll, Oakland, California	1964-66
Atomic Energy Commission	1964-present
Member of Advisory Committee to California State Department of Public Health on Human Use of Radioactive Materials	1967-present

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KENNETH R. McCORMACK, M.D.

BIBLIOGRAPHY

1. Bryant LR, Seki S, Danielson GK, McCormack KR, Trainkle JK: Influence of thoracotomy and intrathoracic dissection on regional pulmonary blood flow. J Surg Res 8:293, 1968.
2. Vaeth JM, Green JP, McCormack KR, Meurk ML: Frontiers of Radiation Therapy and Oncology. The relationship of time and dose in radiation therapy of cancer. 1968, Vol.3, J.M. Vaeth, editor. (Book-Form, available at Tumor Institute, Mt. Zion Hospital & Medical Center Library).
3. Earll JM, McCormack KR, Nusynowitz ML, Forsham PH: Thyroid function in active acromegaly. Military Med 134:47, 1969.
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#907 - MANDL

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

May 23, 1973

Grant Application No. 907

To: The committee comprising Drs. Loosli, Sommers and Wyatt

Subject: Ines Mandl, Ph.D., Columbia University
New application No. 907
"Effect of Smoke Constituents on the Composition of
Connective Tissue Proteins Elaborated In Vitro"

History

This investigator was supported by CTR through Grants 546 and 546A, with renewals, 1966 - 1972.

Application No. 871, "Chemical Composition of Pulmonary Connective Tissue Proteins in Health and Obstructive Lung Disease" was denied in September 1972.

In November 1972 Mandl submitted an informal inquiry, which became Case No. 155. Attached is Dr. Kreisher's memorandum dated January 3, 1973 commenting on this inquiry. Before further action could be taken, Mandl submitted the attached application.

Application No. 907 requests \$38,717 plus one additional year.

Document Submitted (attached)

Application dated February 15, 1973.

Comment

We have obtained opinions from James Travis on biochemical aspects, and from Richard Holmes (at the DuPont Institute) on tissue culture aspects. These opinions, both in letters dated May 9, 1973, are attached.

Perhaps both of these reviews can be summarized in Travis's words "Good, but not very good".


F.W.N.

FWN:wg
Encl.

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Dr. Loosli
Dr. Sommers
Dr. Wyatt

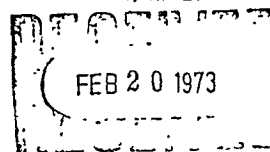
CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022

Application For Research Grant

#907
#871 - denied
#546A-7/69-7/72
#546 -7/66-7/68



Date: February 15, 1973

1. Name of Investigator(s): (include Title and Degrees)

Ines Mandl, Ph.D., Associate Professor of Reproductive Biochemistry

2. Institution & Columbia University College of Physicians & Surgeons

Address. 630 West 168th Street
New York, N.Y. 10032

3. Short Title of Project:

Effect of Smoke Constituents on the Composition of Connective Tissue Proteins
Elaborated In Vitro.

4. Proposed Starting Date:

May 1, 1973

5. Anticipated Duration of this Specific Study:

2 - 3 years

6. Brief Description of Objectives or Specific Aims:

Tissue culture techniques will be used to observe the effect of smoke constituents on isolated cellular elements of the lung and the formation of connective tissue components. Connective tissue growth and molecular integrity is fundamental to the maintenance of the structure of the lung and its normal function. Relatively minor changes in the molecular architecture of the connective tissue proteins may impair resiliency and increase susceptibility to damage by proteolytic enzymes of leucocyte or macrophage origin. It has been observed in several laboratories including our own that the primary damage in obstructive lung disease involves disruption of the elastin framework which appears to be facilitated by familial predisposition of certain individuals coupled with environmental factors such as air pollutants and smoking. Tissue culture offers a unique opportunity to assess the relative contribution of different factors on deviations in composition or structure of bio-synthesized intermediary and end products. The planned study will concentrate on qualitative differences in elastin and collagen on a molecular level. Established lines of diploid fetal fibroblasts and strains of lung epithelial and endothelial cells can be exposed to air pollutants and smoke constituents with only minor effect on growth and respiratory functions. These cells elaborate collagen and elastin under normal culturing conditions and inhibition of key enzymes has been shown to alter biosynthesis, secretion and final composition. The effects of smoke constituents on these parameters have not previously been studied, in part because methods of analysis were not sufficiently sensitive. Preliminary experiments indicate that such a study is now feasible. Recognition of mediators of biosynthesis of forms of elastin with diminished resistance to subsequent damage may allow modifications which would eliminate or neutralize the noxious effects.

7. Give a Brief Statement of your Working Hypothesis: Deviations in the molecular architecture of the susceptible tissue elements may predispose to disruption of the collagen-elastin framework of the lung in emphysema and related diseases. Such molecular defects may be due to genetic or environmental factors. In vitro culture techniques offer unique opportunities to assess the effects of smoke constituents on compositional and structural integrity of the connective tissue proteins produced by cellular biosynthesis.

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8. *Details of Experimental Design and Procedures: (Attach Separate Pages)*

Attached, pages 5 to 9

9. *Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)*

The principal investigator's laboratories on the 4th floor of the Francis Delafield Hospital Division of Columbia University College of Physicians & Surgeons are adequate and fully equipped to carry out all the procedures and analyses described in this proposal.

10. *Additional Requirements:*

A second amino acid analyser is at our disposal in the Department of Biochemistry and a Beckman sequenator in the Department of Medicine. For the culturing of cell strains and isolated lines, Dr. Mary Parshley's complete facilities for growth and maintenance as well as photomicroscopy and time lapse photography will supplement our own.

() *Biographical sketches of all principal and professional personnel (append)*

Attached, page 10

12. *List of publications: (Five most recent as pertinent) (append)*

Attached, page 11

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3.

13. Budget (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Ines Mandl, Ph.D.	25	
Stephen Keller, Ph.D.	20	
Carlton E. Blackwood, Ph.D.	10	
Mary S. Parshley, Ph.D.	10	
Obligatory Fringe Benefits (22% I.M., M.P.; 13% S.K., C.B.)		
Technical		
Yvonne Hosannah, M.S. Biology	75	
Mabel Wong, B.S. Chemistry	50	
Elizabeth Culbert, Secretary	15	
Obligatory Fringe Benefits (13%)		
Sub-Total		
B. Consumable Supplies (list by categories)		
Media, Tissue Culture Supplies		1,000
Glassware, Columns, Parts		1,000
Chemicals, Sephadex, Solvents		1,000
Sub-Total		3,000
C. Other Expenses (itemize)		
Equipment Repair & Maintenance		500
Travel to Scientific Meetings		400
Journal Subscriptions, Xeroxing		200
Sub-Total		1,100
D. Permanent Equipment (itemize)		
None		0
E. Overhead (15% of A+B+C)		5,050
Total		\$38,717

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	3,000	1,100	0	5,405	41,437
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature

Director of Project

Ines Mandl, Ph.D.

Telephone

Signature

Business Officer of the Institution

F.P. Putney, Ph.D.

Telephone

FEB 15 1973 R

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Pulmonary Biochemical Characteristics in Respiratory Distress Syndrome of the Newborn.	NIH SCOR (Scope E of HL 14218 Neonatal Lung Center L.S. James, M.D., principal investigator).	31,222 p.a. 1972/3	6/1/71 - 5/31/76

Pending

Chemical Composition of Lung Connective Tissue Proteins	NIH Program Project Grant (Chemical Predisposition to Lung Injury. G.M. Turino, principal investigator).
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(8) Details of Experimental Design and Procedures.

To accomplish our aim of assessing the effect of various additives on the composition and structure of elastin elaborated by fibroblasts as well as endothelial and apithelial cells of the lung, the experimental design involves (a) culturing of the cells under optimal conditions and in the presence of individual smoke components; (b) isolation of elastin from the cells and from the culture medium and (c) determination of overall composition, sequences and primary structure of the elastin molecules synthesized by the cells. It is expected that results obtained will reflect the changes produced in vivo in individuals exposed to these agents over periods of years or in experimental animals treated with higher doses over shorter intervals. Evidence is accumulating that qualitative abnormalities in lung elastin predispose to disruption of the connective tissue framework and subsequent functional impairment. Such a relationship was suggested as early as 1961 by Pierce et al. (Ann. Int. Med. 55:210, 1961) for patients with primary pulmonary emphysema. More recently, Johanson et al. (J. Clin. Investig. 51:288, 1972) and Hoffman et al. (J. Appl. Physiol. 33:42, 1972) confirmed the association of elastin destruction in the lung with emphysema. At the same time, comparative analyses performed in our laboratory showed statistically significant differences in the amino acid composition, the prevalence of peptide sequences and the susceptibility to enzyme digestion between elastin isolated at autopsy from non affected lungs and from lungs of emphysematous subjects or animals in which experimental emphysema had been induced by proteolytic enzyme injection. (see Keller & Mandl, Proteolysis and Pulmonary Emphysema, C. Mittman, editor, Academic Press, 1972, p.251 and Mandl, et al. id. p.439).

The study of human tissues in adequate numbers, though desirable, meets with difficulties of obtaining specimens and documenting histories as well as separation of effects due to age, genetic factors and environmental constituents. Mittman et al. (Chest, 60:214, 1971) reported that amongst persons with intermediate serum levels of proteolytic enzyme inhibitors (heterozygotes), smokers were more likely than non-smokers to develop emphysema, but amongst homozygously deficient individuals, no difference could be discerned. Tissue culture offers an opportunity to overcome ambiguities and difficulties. It complements animal studies and has the added advantage of greater flexibility and economy and since human cells are involved, possibly a more representative model of the human situation.

Several groups before us have used tissue culture models to reveal effects of air pollutants and smoke components on fibroblasts and respiratory epithelium. Decreased rate of growth, morphological changes and impairment of respiratory function were monitored, but to the best of our knowledge no attempts have been made to find qualitative differences in the connective tissue proteins synthesized by the cells. It is well known through numerous studies of collagen biosynthesis by fibroblast strains that the absence or blockage of essential cofactors such as ferrous iron or ascorbic acid or certain enzyme deficiencies may prevent completion of intermediary steps so that the normal sequence is disrupted and under-hydroxylated or non-glycosylated molecules accumulate intracellularly. Enzymes such as proline hydroxylase, lysine hydroxylase, glucosyl or galactosyl transferase and lysyl oxidase may well be inhibited by smoke constituents. Crosslinking is known to be prevented by lathyrogens and by penicillamine affecting respectively formation of aldehyde intermediates from lysine or hydroxylysine and condensation of the aldehydes to aldols or Schiff bases in the crosslinks characteristic of both collagen and elastin. Although collagen biosynthesis has been studied in

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greater detail than that of elastin, many parallel steps have been recognized, the mechanism of crosslink formation of desmosine, isodesmosine and lysinonorleucine has been elucidated and lung cells in culture were shown to synthesize elastin. Naum and Martin (personal communication) have demonstrated elastin in cultures of a cell line derived from peripheral lung tissue of a 3-month old male C57BL mouse and the same investigators are at present trying to define some of the culture variables that affect elastin accumulation. Exploratory tests in our own laboratory have shown that $200-500 \times 10^6$ cells of a newly established fibroblast culture yielded adequate amounts of elastin to allow isolation of highly purified elastin from both the medium and the cells. This means that enough cells to define compositional and structural differences can be produced at reasonable cost and with available facilities.

We have also explored the feasibility of growing cells in cultures into which toxic substances have been introduced. A considerable literature exists on the effects of air pollutants and smoke constituents on death rate, morphological and functional changes. Indications are that cells in culture may be exposed to substances of considerable cytotoxicity for prolonged periods if concentrations are sufficiently low. Extensive studies conducted by the Leuchtenbergers include monitoring of the effect of tobacco smoke on the growth characteristics of the respiratory epithelium (Cancer Res., 29:862, 1969; Exp. Cell Res., 62:161, 1970; Nature, 234:227, 1971; Nature, 241: 137, 1973). Similar techniques were also used by Crocker et al. (Arch. Environm. Health, 10:240, 1965; Cancer Res., 28:906, 1968 and 30:1312, 1970). These authors observed effects of carcinogenic hydrocarbons on the ultrastructure of respiratory rat epithelium cells. Even earlier, Cooper and associates described alterations of growth of L cells exposed to smoke gases (Proc. Soc. Exp. Biol. Med., 110:11, 1962) and Thayer and Kensler (Science, 146:642, 1964) reported inhibition of growth and protein synthesis of KB cells by the gaseous phase of cigarette smoke. Green and Carolin (New Engl. J. Med., 276: 421, 1967) ascribed direct cytotoxic effects of smoke on pulmonary cells to alteration of surface activity due to abnormal metabolic processes of the cells. Time and concentration related responses of rodent tracheal rings exposed to tobacco products in vitro were measured by Donnelly (Proc. Tobacco and Health Conf. 1970, p.127). At the same conference, Sabharwal and Bhalla (Proc. Tobacco and Health Conf. 1970, pp.97 & 128) described investigations of tobacco smoke components on plant cells in tissue culture. They found tissue culture a sensitive and rapid bioassay for benzopyrene which at concentrations of 10^{-8} M induced marked growth within five weeks although higher concentrations proved quite toxic. Unpublished observations by Parshley et al. established that 50,100 and even 200 ppm of NO_2 introduced into culture media of human and rat lung epithelium by dissolving known amounts of NaNO_2 in an adaptation of the procedure of Rounds and Bils (Arch. Environm. Health 10:251, 1965) had little or no effect on cell count, growth rate or glycolysis. Large vacuoles appearing in the area of the Golgi apparatus indicated a possible effect on lipid metabolism. In another series of experiments, the respiratory poison HCN added to culture media in the form of KCN showed more drastic effects; 20 - 60 ppm inhibited growth by 80-100% on the third day, however, by day 7 normal cells began to recover. As expected, KCN stimulated glucose utilization and lactic acid production although the growth rate was decreased. Based on these results by us and by others, no undue difficulties are anticipated in obtaining cells with sufficient capability to synthesize connective tissue proteins after exposure to specific toxic components of tobacco smoke. Such exposure may change the relative proportion of elastin, collagen and mucopolysaccharides in analogy to the recently demonstrated increases in the production of soluble collagen and glycoprotein by scleroderma fibroblasts

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(Leroy, J. Exp. Med. 135:1351, 1972) or it may affect amino acid composition to reflect the differences observed in vivo between elastin derived from individuals with distinct histories of smoking and of lung disease. The specific experimental protocol proposed for the three phases of this project follows:

(a) In vitro production of fibroblasts and epithelial cells under normal conditions and in the presence of smoke components:

The potential for synthesizing collagen, elastin and glycoproteins may be inherent in several cell types in lung parenchyma. Alveolar epithelial cells, endothelial cells and alveolar macrophages have greater capacities than cells of the same type in other parts of the body. Human fetal lung fibroblasts have been reported by Elsdale and Foley (J. Cell Biol. 41:298, 1969) to have markedly greater potential for production and organization of layers of cells and collagen in tissue culture than gut fibroblasts. It is therefore planned to include in the proposed study cellular elements of the lung and single cell types and to establish primary cultures of explanted small cubes of whole lung as well as monolayer cultures of pure strains of cells originating from a single cell of one type. The organ culture technique has been used by others, e.g. Crocker, Donnelly and the Leuchtenbergers (l.c.) to observe effects of air pollutants including tobacco smoke on growth characteristics of respiratory epithelium. Established strains of epithelial cells from fetal and adult human and other mammalian lung tissues are maintained in our laboratories in connection with other programs. They include the widely used diploid human fibroblast line Wi-38, established by Hayflick (Exp. Cell Res. 25:585, 1961), mouse fibroblasts 3T3 and 3T6, originally obtained from Dr. Howard Green and applied extensively to studies of collagen biosynthesis and the PR 105 strain developed by the Priests which gives high yields of connective tissue proteins. All of these elaborate elastin and will be compared. In addition, we have recently isolated a fibroblast strain which has not been well characterized as yet but produces relatively large amounts of elastin both intracellularly and secreted into the medium. Intracellular elastin can be visualized with specific stains and elastin in the medium monitored by paper chromatographic confirmation of the unique desmosine + isodesmosine spot at the origin. Elastin has been isolated from both cells and media as described under (b).

Lines of epithelium, endothelium and fibroblasts will be prepared by enzymatic digestion and mechanical isolation. Brief trypsin treatment releases predominantly fibroblasts which tend to overgrow other types of cells when planted in culture. Sections of lung will be washed with physiological solution, minced into small pieces, suspended in 0.25% trypsin and stirred 1 hr. Fibroblasts will then be freed and after centrifugation at low speed and removal of the supernatant, the cells will be resuspended at a concentration of 10^6 cells/ml in 10% fetal calf serum in Ham's medium F12 or 20% human placental serum in Parker medium 199 and incubated at 37°C in Carrel flasks or milk dilution bottles. To obtain epithelial cells in almost pure culture, the connective tissue elements are digested by collagenase. Following the trypsin treatment, the cells are resuspended in a 0.1% collagenase solution, spun for two more hours, filtered and planted. Isolated colonies which develop on the floor of flask cultures from one or more of the same type can be scraped off and subcultured. From these pure cultures lines originating from one cell may be obtained by digestion, suspension and dilution to not more than 100 cells per ml. The few colonies or clones which develop around these cells can then be isolated mechanically and subcultured. To study the effect of smoke components, different concentrations of selected constituents will be introduced into the medium. Benzopyrene, 10^{-6} M to 10^{-9} M will be investigated; nitrite will be dissolved in the culture medium in the form of NaNO_2 which was shown to produce a death rate of cells comparable to that of direct exposure to NO_2 gas. In the

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latter case, the cell suspension will be inoculated first and after the cells have become attached to the vessel floor, the medium will be changed to introduce NaNO_2 in dosage ranges from 0-200 parts per million. Other individual components will be selected later in the program and introduced in a similar manner.

Tobacco smoke is said to contain at least 10,000 different substances. The components believed to be most responsible for the alleged toxic effects are polycyclic aromatic hydrocarbons. To confirm this impression and eliminate other constituents, fractionation of smoke condensations may be useful. We intend, therefore, to follow the fractionation procedure recently described by Bresch et al. (Proc. Soc. Exp. Biol. Med. 141:747, 1972) and assess the effect of some of the fractions on elastin biosynthesis. Smoke condensate will be dissolved first in methanol to remove polar substances and extracted twice with cyclohexane. The combined extracts will then be extracted twice with nitromethane to separate aliphatic compounds from unsaturated compounds, heterocyclic components and aromatic substances. The nitromethane phase will contain almost all the benzo-pyrene plus traces of different phenols and nicotine, although most of the nicotine will have been left in the methanol fraction. In order to render the components enriched in the nitromethane phase soluble in water and thus facilitate their incorporation in aqueous media, the authors bound it to ovalbumin. More than 90% of the residue bound to ovalbumin was removable by benzene resulting in enrichment of benzopyrene and other aromatic hydrocarbons which bound to ovalbumin. Further purification can be achieved by chromatography of the ovalbumin adduct dissolved in phosphate buffer on Sephadex G 150.

(b) Isolation of elastin from tissue culture cells and media:

Methods successfully applied to the isolation of elastin from human and animal lung parenchyma have been adopted to allow extraction of elastin from used up growth media and from the viable cells. Extraction with NaCl and lipid removal with butanol and acetone will be followed by boiling in hot alkali. This process has been shown to destroy collagen and associated proteins and should result in very pure elastin. Nevertheless, the procedure will be complemented by milder, less drastic techniques to guard against possible loss of incompletely crosslinked or altered elastin which may be partly soluble in NaOH . Some aliquots will be subjected to digestion with pancreatic elastase which will yield elastolytic breakdown products which can be analysed. Others may be subjected to collagenase which will leave the elastin intact and, at the same time, indicate the normalcy or otherwise of collagen produced. Newly formed elastin has also been reported to be closely associated with structural acidic glycoproteins. Urea-mercaptoethanol is said to dissociate the complexes and will therefore be added to a representative proportion of the tests. Presence of desmosine and isodesmosine and the high proportion of valine and virtually no hydroxyproline will prove that elastin is involved.

(c) Determination of compositional or structural differences in elastins synthesized under normal conditions and in the presence of potentially toxic smoke components:

It is our contention that the relative amounts of collagen and elastin are less revealing of fundamental changes in the connective tissue framework than chemical composition and sequences within the molecule, and we intend to focus on these parameters. For complete amino acid analyses on a Technicon amino acid analyzer, the connective tissue proteins will be subjected first to hydrolysis in 6N HCl at 110°C for 24 hrs. In addition to the presence of the unique amino acids,

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desmosine and isodesmosine, the most characteristic feature of elastin is the large percentage of valine, 13.5 residues per 1000 in bovine ligamentum nuchae elastin, seven times as much as in collagen. Differences in valine and other non-polar amino acid residues and resulting shifts in the relative percentage of neutral, basic and acidic amino acids in the isolated elastins will be recorded and relationships sought with developmental stages of elastin biosynthesis. In the collagen fractions, changes in the total amino acid composition, polar residues and hydroxyproline content will be studied.

To reveal differences in the amino acid sequences, aliquots of purified elastin from the same specimen will be subjected to enzymatic hydrolysis with pancreatic elastase and microbial elastases and to limited breakdown with alcoholic KOH and collagen will be digested with collagenase and cleaved with CNBr. The peptide products will be separated by fractionation on DEAE and SE-Sephadex and Bio-Gel P₂, Dowex 50 and various Sephadex columns. The elution profiles obtained will be compared and the combined fractions under each peak analyzed. N-terminal amino acids will be determined by Edman degradation, C-terminal residues by hydrazinolysis. Since preliminary experiments have indicated that regions enriched in desmosine are poor in valine, non-specific microbial proteases with high elastolytic activities which preferentially cleave valine and isoleucine containing sequences, e.g., thermolysin, will be used for further breakdown and sequential analysis of the neutral regions of elastin. For both collagen and elastin analyses, microsomal hog kidney aminopeptidase, which preferentially liberates dipeptides of the Gly-Pro type from Gly-Pro-X sequences (Hopsu-Havu and Glenner, Acta Chem. Scand. 22:299, 1968) and an E. Coli peptidase which releases N-terminal non-polar amino acids from sequences with proline in the penultimate position will also be applied. Special attention will be paid to differences in the location and distribution of unusual components, known crosslinking amino acids and valyl peptides and the ratios of polar to non-polar amino acid residues in each fraction.

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(11) Biographical sketches of investigators:

Dr. Ines Mandl, R, received her early education in Cork, Eire and her M.S. (organic chemistry) and Ph.D. (protein chemistry) at the Polytechnic Institute of Brooklyn in R and R respectively. From 1945-1949 she worked as assistant and coworker of the late professor Carl Neuberg at New York University. In 1949 she joined Columbia University, Department of Surgery as research associate and chief chemist of an army-sponsored project on bacterial collagenases. Thereafter she was assigned to the Department of Microbiology and since 1959 the Department of Obstetrics and Gynecology. From 1956 to 1972 she has been Assistant Professor of Biochemistry and principal investigator on several government and non-government sponsored projects. Recently she has been promoted to Associate Professor of Reproductive Biochemistry. She is a member of numerous professional societies and to date author or coauthor of 77 original or review articles and 47 abstracts of papers presented at scientific meetings. Her main research interests are the relationship of structure and function in connective tissue proteins and specific enzymes degrading the proteins. She is also Editor-in-Chief of Connective Tissue Research: An International Journal.

Dr. Stephen Keller, R is at present research associate in Biochemistry and in Obstetrics & Gynecology at Columbia University College of Physicians & Surgeons. He obtained his Ph.D. in Biochemistry at Rutgers University in R, his M.A. in Physiology from St. John's University in R and his B.S. in Chemistry at the City College of New York in R. From 1955 to 1959 he worked as a fellow in Biochemistry at the Boyce Thompson Institute in Yonkers, New York, under the direction of Dr. Richard Block. Since 1959 he has been in the principal investigator's laboratory at Columbia University. His main interests evolve around physicochemical analyses of elastin moieties. He is experienced in protein and enzyme fractionation techniques and co-authored chapters in the Laboratory Manual of Analytical Methods of Protein Chemistry, edited by Alexander and Block as well as 33 research papers.

Dr. Carlton E. Blackwood, R, is at present research associate at the College of Physicians and Surgeons and also Associate Professor of Biology at Iona College, New Rochelle, New York. He obtained his B.S. in Chemistry at Long Island University in R attended Medical School at Montpellier University, France, from R, then returned to graduate work at New York University where he obtained his M.S. in Biology in R and his Ph.D. in Biology in 1962. Between 1954 and 1959 he worked at the Funk Foundation for Cancer Research and, since 1959, at Columbia University College of Physicians & Surgeons. His main interests revolve around studies of proteolytic enzyme systems in tissue cultures, animal models and human tissues. To date he has co-authored 17 published papers or abstracts.

Dr. Mary S. Parshley, REDACTED, is at present Assistant Professor of Anatomy assigned to the Department of Obstetrics & Gynecology. She obtained her A.B. from Smith College in R and M.A. and Ph.D. degrees in Anatomy from the University of Pennsylvania in R and R respectively. She has been associated with Columbia University College of Physicians & Surgeons since 1940 and is a recognized authority in the field of tissue culture. Her 43 publications to date include papers on tissue culture of adult tissues, studies of the behavior of normal and malignant cells in tissue culture under diverse conditions, and the establishment and maintenance of tissue culture cell lines.

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(12) List of publications (five recent, most pertinent).

1. Turino, G., Senior, R., Garg, B. D., Keller, S., Levi, M. and Mandl, I.: Serum Elastase Inhibitor Deficiency and Alpha₁-antitrypsin Deficiency in Patients with Obstructive Emphysema. *Science*, 165:709, 1969.
2. Mandl, I., Keller, S., Hosannah, Y. and Blackwood, C. E.: Induction and Prevention of Experimental Emphysema. In: *Pulmonary Emphysema and Proteolysis*. Ed. by C. Mittman, Academic Press, 1972, p.439.
3. Keller, S. and Mandl, I.: Qualitative Differences Between Normal and Emphysematous Human Lung Elastin. In: *Pulmonary Emphysema and Proteolysis*. Ed. by C. Mittman, Academic Press, 1972. p.251.
4. Evans, H. E., Mandl, I. and Keller, S.: Respiratory Distress Syndrome: Serum Enzyme Inhibitor Levels and Lung Tissue Elastin Composition. In: *Pulmonary Emphysema and Proteolysis*. Ed. by C. Mittman, Academic Press, 1972. p.91.
5. Mandl, I., Keller, S. and Levi, M.: The Relationship Between the Antigenicity and Chemical Composition of Components of Elastin Digests. In: *The Chemistry and Molecular Biology of the Intercellular Matrix*. Vol.I, p.657, E. A. Balazs, Ed., Academic Press, 1970.

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GROVE G. WILEY, M.D.
T. TIMOTHY MYODA, PH.D.
MICROBIOLOGY

PAUL B. HAMILTON, M.D., PH.D.
RICHARD HOLMES, PH.D.
BIOCHEMISTRY

May 9, 1973

G. DEAN MACEWEN, M.D.
MEDICAL DIRECTOR AND
SURGEON-IN-CHIEF

Dr. Frederic W. Nordsiek
Associate Scientific Director
The Council for Tobacco Research -- U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Re: Your Grant Application #907

Dear Dr. Nordsiek:

The program of research titled, "Effect of Smoke Constituents on the Composition of Connective Tissue Proteins Elaborated In Vitro," proposed by Dr. Ines Mandl and associates of Columbia University College of Physicians and Surgeons is very ambitious. The project covers a considerable number of parameters in the fields of Tissue Culture, Protein Chemistry and Preparation of Chemical Constituents of Tobacco Smoke.

In reviewing this proposal, I have divided the approaches suggested by Dr. Mandl into a number of headings. I will attempt to comment on aspects of each heading. The headings selected are:

- a) The use of Tissue Culture to Study Protein Production.
- b) The Effect of the Environment on Cells -- particularly as affected by the addition of chemicals -- as in smoke.
- c) Aspects of Protein Chemistry -- particularly protein composition and structure.

It seems to me that the potential results that may be obtained from this project could be quite significant. While the course of development of the project will obviously rest heavily on the prime investigator, there is no question in my mind as to the basic capability of the investigators in this direction.

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May 9, 1973

When the project, if undertaken is finished, it should be possible to determine the effectiveness of Tissue Culture as a tool in this area of research. The effect of cross linking of natural structural polymers such as elastin and collagen as regards function and aging reaches back historically in the field of biochemistry. The effect of the current environment that man is exposed to is certainly pertinent. This could develop into a very interesting series, particularly if the wider aspects along with the necessary documented experimental details are kept in perspective.

Yours very sincerely,



Richard Holmes, Ph.D.
Associate Chief of Biochemistry

RH:ejm

Enclosure

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(a) Tissue Culture and Protein Production.

Cell cultures are conveniently divided into two categories (A) Primary cells isolated from fresh tissue and (B) Established cells which may be cultured indefinitely. In order to grow primary human cells successfully in Medium 199 or Ham's F12 serum must be added. The use of both these media has been suggested by Dr. Mandl along with added fetal calf serum or human placental serum. It should be noted that both types of sera are abnormal compared to the environment in which fibroblasts and epithelial cells are found in the lung. The proteins, hormones, particularly steroid hormones and a variety of small molecular substances are different, many unrecognized qualitatively and quantitatively. All these substances exert a profound effect on cellular metabolism. For this reason one might suggest that any sera used in tissue culture be monitored for proteins by acrylamide gel electrophoresis and by isoelectric focusing. Lipids can be monitored by thin layer or gas chromatography. That cell cultures take up proteins from the environment has been documented by Hamburger et al (1) and Ryser (2). Cells apparently adsorb and remove proteins from the medium particularly albumin. Dr. Mandl suggests oval albumin for stabilizing and dispersing chemicals found in smoke. The use of any albumin for this purpose requires some consideration of its chemistry, a brief reference to King (3) and Foster(4) reports on albumin will indicate the factors involved.

Much work in the field of tissue culture has been directed towards maximum stimulation of growth and the elimination of inhibitory or toxic substances. This point of view probably accounts for the extensive use of fetal calf serum, since it lacks many globulins and also the large lipoprotein fraction found in the adult. A case in point that the composition of the serum directly effects the metabolism of human cells is the recognition of so-called Rily bodies (5) in cells grown in fetal calf serum but not when cells are grown in human serum. These bodies are associated with certain glycoprotein disorders. Maximum rates of growth while convenient may be abnormal and lead to unbalanced metabolism. One might suggest that consideration be given to fully chemically defined medium such as described by Bakken et al (6) and Holmes et al (7). Established human cells may be grown as monolayers without difficulty and primaries may be grown out to confluency. That trypsin, used in the transfer of cell cultures, alters the nature of the cell membrane is known and recently documented by Burger (8). During this period before the cells have re-established their outer coat they may be affected by ingredients in smoke. The course of elastin or collagen production during growth from the time of inoculation to the time of confluency may be different. Dr. Mandl has indicated the role of ascorbic acid which raises the whole question of oxidation and reduction and the production of free radicals as related to the cross-linking of proteins. The role of lipoproteins, for example isolation and characterization of Lung surfactant by Steim (9) may have a significant effect on transport and uptake of chemicals.

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(b) The Effect of Environment on Cells.

Many aspects of the environment as it affects cells could be discussed. Perhaps a pertinent aspect is the manner in which tobacco smoke ingredients are incorporated in the medium and some of the components in culture medium which may be affected. Many chemicals do not always enter cells readily and can be influenced by a second substance such as a carrier (albumin), a hormone (insulin) or one that affects ionic relationships (polylysine or polyglutamic), surfactants can be involved (phospholipids). In this regard the outer cell barrier (cell membrane) can be involved (see previous section). Alterations that are induced may result in changes from the limited growth potential of primaries to the unlimited growth of established cells. Under these circumstances it is not unreasonable to speculate that protein structure may be significantly altered.

Ascorbic acid, already commented upon in relation to oxidation-reduction, raises consideration of other reducing materials often used in culture media. The list of substances includes DPN, TPN, glutathione, cysteine (as opposed to cystine), lipoic (thioctic acid), alpha tocopherol and others. These materials, for example DPN (oxidized and reduced) are probably involved in the deamination and aldehyde condensation of lysine involving cross-linking of proteins, which Dr. Mandl proposes to examine. Glutathione has recently been shown by Meister (10) to be involved in transport, a cyclic system has been proposed. Thioctic acid introduced in culture media by Holmes was confirmed as a requirement for growth of human cells by Chang (11).

Tween 80 has been used in the past when a protein-free system was required, for dispersing hydrophobic substances. Most surfactants are highly toxic in tissue culture and certainly the chemical structure of Tween 80, polyethylene backbone, is not natural. As an alternate, cholic acid, as opposed to desoxycholic, has been proposed and used successfully for preparing lipid dispersions in tissue culture (7).

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(c) Protein Structure

This aspect is the highlight of Dr. Mandl's approach. It will be very interesting to discover if desmosine and isodesmosine linkages are affected by the environment since cystine in elastin appears to be constant at one half residue. Specifically, one may look for disturbances in the metabolism of lysine. While the insolubility of elastin limits electrophoretic studies this may not apply to enzymatically prepared fragments and indeed Dr. Mandl suggests looking at such fragments. Some criterion of purity of the fractions analysed will have to be developed in order to arrive at an understanding of the microheterogeneity of both elastin and collagen. Oxidation-reduction relationships in the cellular environment may affect condensation of lysine to desmosine and isodesmosine and to the formation of lysine nor leucine and merodesmosine. In this regard Paz et al's (12) report on the presence in elastin of possible cyclic precursors of desmosine and isodesmosine may be significant. Within a single culture the point at which cells are harvested early log phase or at confluency in the case of primaries could be relevant. With primaries which exhibit a limited life span of 60 to 65 cultures, the nature of protein in actively dividing cultures (6-8 subcultures) may be different to that found in older cultures (50-60 subcultures) (see Hayflick). Some relative changes in enzyme coding resulting in different Km values appears to occur as cells age according to Holliday and Tarrant (13). The same may also be true of elastin and collagen in cells as they age normally and different in cells under the influence of some of the ingredients of smoke. It would seem likely that the age of the tissue donor may affect the results, again as suggested by Hayflick. Other linkages in protein should not be ignored, for example di- and tri-tyrosine as indicated by LaBell et al (14).

In summary, it is conceivable that the expansion of knowledge of structure of both elastin and collagen could occupy the full time of the investigators. A balance will have to be struck between examining the effects caused by the environment and the structural studies.

(NOTE - While this report was in preparation, two current pertinent articles appeared which are worth mentioning. The first by Tanzer on "Cross linking of Collagen" in SCIENCE, May 11, 1973. The second by Blumenkrantz, et al on "Effect of Amino Acids on Collagen Biosynthesis", IN VITRO, Vol. 8, No. 5, March-April 1973).

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(1) Hamburger, R. N., D. A. Pious and S. F. Mills, 1963, Immunology 6: 439.

(2) Ryser, H. J. P. 1968, Science 159: 390

(3) Sogami, M., H. A. Petersen and J. F. Foster 1969, Biochemistry 8: 49.

also

Sogami, M. and J. F. Foster 1962, J. Biol. Chem. 237, 2514.

(4) King, T. P. and Spencer, E. M. 1970, J. Biol. Chem. 245, 6134.

(5) Danes and Bearn 1966, J. Exp. Med. 123, 1.

(6) Bakken, P. C., Evans, V. J., Earle, W. R. and Stevenson, R. E. 1961,

Am. J. Hyg. 76, 96.

(7) Holmes, R. 1967, J. Cell Biol. 32, 297.

(8) Burger, M. M. 1973, Fed. Proc. 32, 91

(9) Stein, J. M. 1969, Biochem. & Biophys. Res. Comm. 34, 434

(10) Meister, A. 1973, Science 180, 33.

(11) Chang, R. S. and Humes, H. 1926, J. Exp. Med. 115, 937.

(12) Paz, M. A., P. M. Gallop, O. Olga Blumenfeld, E. Henson and S. Seifter

1971, Biochem. & Biophys. Res. Comm. 43, 289.

(13) Holliday, R. and G. M. Tarrant 1972, Nature 238, 26.

(14) LaBella, F., F. Heeley, S. Vivian and D. Thornhill 1967, Biochem. Biophys.

Res. Commun. 26, 748.

(15) Tanzer, M. L. 1973, Science 180, 561.

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THE UNIVERSITY OF GEORGIA

Department of Biochemistry

BOYD GRADUATE STUDIES RESEARCH CENTER

ATHENS, GEORGIA 30601

TELEPHONE 404 542-1114

May 9, 1973

5/17/73
Acquired
in-house
file
JCT

Dr. Frederic W. Nordsiek
Associate Scientific Director
The Council for Tobacco Research - U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Dear Dr. Nordsiek:

Enclosed are my comments regarding the proposal of Dr. Mandl.
If you have any other proposals which you feel I might be qualified
to review, please do not hesitate to send them to me.

I am planning to attend the International Congress of Biochemistry
in Stockholm, Sweden and would like to use some of the travel funds
in my Tobacco Grant for this trip. Would you be so kind as to send
me a letter authorizing the use of travel funds for foreign travel.
I believe you have no objection to using travel funds for foreign
travel, if my memory is correct, but our business office requires
some type of letter from you.

Yours sincerely,

James Travis
James Travis
Associate Professor

JT/mb

Enclosure

1003539766

Review of Proposal No. 907

Dr. Mandl proposes to culture fibroblasts from several cell lines in both the presence and absence of smoke constituents, to isolate elastin from the tissue cultures, and to investigate the comparative compositions of the elastins isolated by amino acid analysis and sequential analysis. She suggests that these analyses will give some insight into the effect of smoke constituents on the types of elastins elaborated and may give indications as to possible damage to specific enzymes required for the formation of the complex elastin molecule.

While the overall ideas suggested by Dr. Mandl are good, I question whether she is really prepared to perform the proposed experiments. The immediate problem would be whether there are enough human cell lines available for culturing. This is not clear from the proposal as it skips back and forth from humans to other animals and I seriously question whether one can compare mouse elastin with human elastin. I will admit, however, that the effects of smoke constituents on the composition of elastin from an individual cell line would make a very interesting and revealing study.

This brings me to another important point. It is obvious that the Principal Investigator is biting off far more than she can chew. Her productivity has not been good in the past few years (note the references to three un-refereed papers in "Pulmonary Emphysema and Proteolysis") and she should be concentrating on a much smaller area.

Finally, just because a sequenator is present in a building does not mean it is available for use. Furthermore, the isolation of peptides is an arduous task and none of her colleagues appear to have much experience in peptide sequencing.

SUMMARY: While this is an interesting proposal I feel the Principal Investigator should restrict herself to a much smaller aspect of the problem, at least initially. I recommend that if funds are available she be given about \$20,000 for the first year. She certainly needs no more. Frankly, the budget is written as if she were the general of an army fighting a battle against emphysema.

Rating: Good, but not very good.

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#841A-MITMAN

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

July 27, 1973

Grant application No. 841A
CHRONIC PULMONARY DISEASE

TO: The committee comprising Drs. Jacobson, Loosli and Wyatt

SUBJECT: Charles Mittman, M.D., City of Hope Medical Center, Duarte
Continuation application No. 841A (no commitment)
"Hereditary Susceptibility to Bronchitis-Emphysema"

History

Grant #841, for \$35,000., was awarded (without assurance of continued support) for 1972 - 1973.

Application #841A requests \$36,608. plus one additional year.

Documents Submitted (attached)

1. Application dated July 20, 1973 (19 pages).
2. Progress Report #1, October 1, 1972 to May 31, 1973.
3. "PULMONARY DISEASE QUESTIONNAIRE"
4. Abstract "CHRONIC BRONCHITIS RISK IN COKE OVEN WORKERS" by Mittman, Lewis and Lieberman, CLINICAL RESEARCH 21: 668, 1973.

Comment

Attached are copies of the following staff memos:

1. Dr. Gardner, June 20, 1973 re: Progress Report #1.
2. Dr. Hockett, June 28, 1973 re: Visit to CTR office by Mittman June 27, 1973.
3. Dr. Gardner, July 25, 1973 re: Site visit to Mittman July 20, 1973.

FWN:gh

FWN
F.W.N.

Attachment

1003539769

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET

NEW YORK, N. Y. 10022

(212) 421-8385

Application for Research Grant

(Use extra pages as needed)

JUL 26 1973

Date: July 20, 1973

1. Principal Investigator (give title and degrees):

Charles Mittman, M.D., Director, Department of Respiratory Diseases
Jack Lieberman, M.D., Associate Director, " " "

2. Institution & address:

City of Hope Medical Center
1500 East Duarte Road
Duarte, California 91010

3. Department(s) where research will be done or collaboration provided:

Department of Respiratory Diseases, City of Hope Medical Center;
Medical Department, Kaiser Steel Corporation, Fontana, CA

4. Short title of study:

Hereditary Susceptibility to Bronchitis-Emphysema

5. Proposed starting date:

October 1, 1973

6. Estimated time to complete:

Two years

7. Brief description of specific research aims:

See attached pages for items 7 through 13

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7. Brief description of specific research aims

Investigators have long suspected that inheritance can influence an individual's susceptibility to the damaging effects of his environment. Examples of genetic-environmental interplay leading to disease are accumulating in various fields of medicine. Such diseases are particularly important since they can be prevented if the genetic predisposition can be recognized prior to injury and the pathological process can be aborted by correction of the underlying defect or modification of the environmental factors. Data are accumulating which suggest that α_1 -antitrypsin deficiency leads to chronic obstructive lung disease (COLD) through such a mechanism. This project seeks to develop the information needed to devise preventive programs for these diseases by:

- 1) Continuing the studies successfully initiated at the Kaiser Steel Mill, Fontana, California. Preliminary results suggest that information about a worker's inheritance can be used to make job placement recommendations which will benefit his future health.
- 2) Investigate the possibility of extending this study to steel mills in other locations so as to assess the role of climate, air pollution and other factors in the effects being observed.
- 3) Further evaluate a simplified, practical approach to detecting antitrypsin abnormalities in large populations.
- 4) Search for other identifiable genetic factors which could predispose to the development of lung disease. We will establish techniques to examine the level of proteases in leukocytes and determine if variations in this factor account for differences in susceptibility to lung disease. Further, patients with lung disease apparently related to familial factors will be examined in an attempt to identify causative genetic factors other than antitrypsin deficiency.

8. Working hypothesis

Interactions of genetic and environmental factors are involved in the etiology of a significant fraction of cases of chronic obstructive lung disease. α_1 -antitrypsin deficiency is one factor which predisposes to COLD by this mechanism. By studying industrial workers and selected patients we can 1) evaluate the importance of antitrypsin deficiency as a predisposing factor, 2) search for other identifiable inherited factors, 3) develop efficient means for screening large populations to identify individuals with genetic variants of α_1 -antitrypsin, and 4) assess the role of various industrial, atmospheric and personal air pollutants in the genesis of clinical lung disease in genetically predisposed individuals. Such information can lead to the establishment of effective obstructive lung disease prevention programs.

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9. Experimental design

Surveys will be continued at a plant of the Kaiser Steel Corporation located near the City of Hope Medical Center. This site has been selected because:

- a) the industrial health department, management and labor are interested in this project and are cooperating fully with it.
- b) the 5000 man work force is relatively stable.
- c) the plant is an integrated metal mill and its various operations involve a wide range of dust, fume and smoke exposure levels. Large numbers of workers have had prolonged employment in various areas with well defined irritant levels (as coke oven workers, welders, grinders, aluminum workers).
- d) workers who have been forced to retire or change jobs because of respiratory and other symptoms can be traced. Their detailed work records are available, and for those receiving medical care through the local Kaiser Health Plan, accurate medical histories can be obtained.
- e) it is possible to bring selected subjects to the City of Hope Medical Center for further studies.

Blood samples are obtained and data collected by questionnaire at the plant. Standardized methods are used to obtain information on 1) work history and occupational and avocational lung irritant exposure now and in the past; 2) air pollution exposure at home and while commuting; 3) personal cardiopulmonary symptoms and smoking history, and 4) family history of cardiopulmonary disease, smoking habits of relatives with disease and ethnic background data.

Blood samples are analyzed for antitrypsin abnormalities (see below) and selected subjects are brought to the Medical Center for extensive studies of lung function in order to determine the following:

- 1) what abnormalities are seen in subjects with protease inhibitor variants as compared to those with the normal Pi phenotype. To isolate the independent influence of the protein abnormality, subjects are matched by age, sex, ethnic background, smoking history, occupation and other pertinent characteristics. Appropriate statistical methods are employed for data analysis.
- 2) do smoking, industrial air pollution exposure, place of residence and other factors particularly influence the lung function of subjects with various Pi types.
- 3) are individuals with a family history of lung disease particularly susceptible to lung disease without regard to Pi type.
- 4) which tests are most sensitive to early changes in lung function or structure and what significance do these changes have.

The test battery carried out at the Medical Center includes the following procedures:

- 1) spirometry with measurement of vital capacity, flow rates and flow-volume curves.
- 2) inert gas dilution and washout for measurement of lung volume, closing volume and ventilation efficiency.

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- 3) diffusing capacity measured by the breathholding and steady state methods.
- 4) body plethysmography for measurement of lung volume and airway resistance.
- 5) rest and exercise ventilation. Arterial or capillary blood gas analysis for calculation of arterial-alveolar gradient can be performed on selected subjects.
- 6) standard chest radiographs.
- 7) scintillation camera lung scan for assessment of regional perfusion.
- 8) electrocardiograms.

Individuals with antitrypsin deficiency are considered to be ideal subjects for a study of the sensitivity of new tests of abnormalities of lung function and structure. The availability of detailed standard pulmonary function test results on deficient and matched control subjects and the opportunity to initiate a prospective study of such subjects will permit evaluation of the significance of abnormalities detected. For example, in addition to measuring diffusing capacity by the standard carbon monoxide steady state and breathholding methods, we use the so-called uptake-washout technique on all subjects. This test employs digital computer methods to examine ventilation and CO gas transfer in terms of a multicompartiment model of the lung. Preliminary results of studies on volunteers with antitrypsin deficiency suggest that this method can reveal evidence of non-uniformity of lung function prior to the presence of abnormalities of overall function.

By sampling the entire work force of this plant, approximately 300 individuals with abnormal antitrypsin variants would be available for detailed study. Of this potential panel of 600 subjects (deficient and control) nearly one-fourth will have been seen at the Medical Center this year. The remaining subjects could be studied, if this is considered worthwhile, over the next 2 to 3 years. During the first year of this project we concentrated our studies on subjects who were deemed likely to yield the most clearcut findings. Studies of the coke oven area, indeed, suggest that our working hypothesis is correct and that information about a worker's inheritance can be used to make job placement recommendations which will benefit his future health. Continuation and expansion of this project is deemed essential to establish if the trends seen in these preliminary data are real.

Extension of These Studies to Other Geographical Areas:

Encouraged by our preliminary results we have initiated discussions to extend these studies to steel mills located in other areas of the country. If the techniques being used can effectively assess the health impact of various hazards and lung irritants, then sampling at different sites might permit us to determine the contribution of climate, residential air pollution and other variables. Preliminary contacts have evoked a favorable response and it is hoped that cooperative protocols can be developed over the coming year.

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Characterization of Pi Phenotype:

Broad application of the findings of this study depends on a practical method for detecting Pi abnormalities in large populations. Pi phenotyping currently requires the use of the slow, tedious and expensive methods of acid starch gel and crossed antigen-antibody electrophoresis. Phenotyping cannot be performed as a primary approach to large numbers of sera. Methods for quantitating the amount or activity of protease inhibitor in serum are simple, inexpensive, reliable and reproducible. They lend themselves to automation and can be applied to large population studies. However, the overlap between the normal range and that seen in phenotypes associated with the intermediate deficiency limits the usefulness of these methods. A two-stage screening procedure appears to be a reasonable compromise between the need for definitive results and the limitations of time and expense.

Two approaches to a screening method have been examined by us. One involves defining limits for the concentration or activity assays which would establish with a high degree of assurance that a blood sample comes from a normal phenotype. For example, serum trypsin inhibitory capacity (STIC) assays can be done easily on large numbers of blood samples. It has been determined that all bloods with an STIC of 0.80 units or less are from individuals with deficient phenotypes. We have also established that 98% or more of blood samples with STIC values over 1.2 units have the normal or MM phenotype, so that such bloods need not be studied in any greater detail than an STIC assay. However, abnormal phenotypes are seen among the samples between these two ranges and such bloods account for nearly half of all samples from healthy subjects. Thus, screening by STIC does not eliminate the need for phenotyping large numbers of sera.

Another approach to a two-stage screening method is more promising. Serum antitrypsin levels can be easily quantitated by the immunodiffusion method. We have established that Pi phenotypes which most commonly yield deficient levels, the ZZ, MZ, SZ, SS and MS variants, give a characteristic double ring pattern on immunodiffusion assay when a special antibody is used. A commercially available method for performing this test will be evaluated in our laboratory during the coming year using bloods from this industrial survey as well as other samples.

Search for Other Genetic Factors:

It is clear that antitrypsin deficiency accounts for only a fraction of cases of lung disease associated with familial factors. Our laboratory has been interested in identifying, if possible, other factors. Defects of the immunoglobulin system do not account for any appreciable fraction of cases, nor do cystic fibrosis heterozygotes occur with any unexpected frequency among patients with chronic lung disease. Galdston, Janoff and Davis have recently suggested that an inherited variation in the level of a leukocytic lysosomal

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protease influences the expression of α_1 -antitrypsin deficiency (Amer Rev Resp Dis 107:718-727, 1973). To evaluate this hypothesis we will establish this method in our laboratory and test our patients and volunteer subjects for such activity. Further, patients with lung disease not associated with antitrypsin deficiency will be tested to determine if excessive quantities of protease could cause disease in individuals with normal inhibitor activity.

Finally, we will continue to survey patients for indications of familial tendencies toward lung disease and attempt to characterize the abnormalities in such patients. Individuals who have developed significant disease at an early age and without the prior exposures usually seen in such patients can offer important clues to underlying predisposing factors. The same can be said for patients who report a strong family history of lung disease. We have been studying intensively such patients seen on our clinical service. During the coming year the principal investigator will have an unusual opportunity while on sabbatical leave in Israel to extend these exploratory studies. Data collected by our laboratory and preliminary data from Israel suggest that antitrypsin deficiency is rare among Jewish and Arab patients with chronic obstructive lung disease. This question will be systematically examined by performing antitrypsin assays and collecting standardized clinical data on approximately 400 patients in the Tel Aviv area. Unusual prevalence rates for lung disease will be sought in various highly inbred population groups residing in that area. We are aware, for example, that early onset of severe bronchitis is common in men in the colony of Samaritan Jews residing in Israel. We have participated in a preliminary cooperative study which suggests that antitrypsin deficiency plays no role here. This will be further examined and the type and degree of lung disease present in such groups will be assessed in the field by spirometry and, as indicated, by more complete tests in a hospital pulmonary function laboratory.

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10. Physical Facilities Available

Laboratory Facilities: The research and support facilities available at the City of Hope Medical Center are more than adequate to carry out the project outlined here.

Lung Biochemistry Laboratory: A fully equipped 1600-square foot laboratory is available to this project. Equipment includes a Gilford Model 2400 spectrophotometer, Radiometer pH stat, Gilford Model 300 microspectrophotometer, Beckman microzone electrophoresis equipment, a Gelscan apparatus, Gelman immunoelectrophoresis and Bucher Starch Gel Electrophoresis equipment, an IL flame photometer, Kahn surface tension balance and refrigerated centrifuge. Facilities include a walk-in cold room, small animal room, animal exposure chamber and hood.

Human Physiology Laboratory: The clinical pulmonary physiology laboratory occupies 1200-square feet. Its equipment includes Collins and Wedge spirometers, treadmill, a pressure body plethysmograph, Radiometer micro blood gas analyzer, electronic analyzers for CO₂, CO, He and N₂, an 8-channel Sanborn recorder, a 7-channel Sanborn analog tape recorder and a laboratory computer (PDP 12) with 8K memory, two digital tape units, hardware arithmetic, 16-channel A to D and D to A converters, CRT and teletype communication device. The laboratory now operates 8 hours per day including week ends in order to accommodate the added work load of this project and to facilitate the study of subjects who are employed full time.

Animal Physiology Laboratory: Although not directly involved in the present project, approximately 200 square feet in the animal laboratory is assigned to the Respiratory Disease Department. Equipment includes a Radiometer blood gas analyzer, an 8-channel E for M recorder, Harvard respirator and electronic analyzers for CO and He gas. The animal laboratory also has radiology equipment available for fluoroscopy and general radiography.

Other Clinical Laboratory Units: Each clinical department has laboratory facilities which, as needed, are available to this project.

Basic Science Laboratories: The City of Hope Medical Center complex includes Basic Science Divisions which house and support the research programs of over 50 PhD investigators. Major research areas include genetics, molecular biology, neurosciences, experimental pathology and immunology. Although not directly involved in this program, these units are available for consultation, collaboration and equipment support. The small relative size of the Medical Center staff and the geographic proximity of all departments contribute to the ease of communication between the medical and scientific staff. Consultation is available on problems of molecular genetics from such investigators as Dr. Susumu Ohno of the Biology Division.

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Biomathematics: The Biomathematics Department offers computer and statistical support and consultation service and serves as a tie to the UCLA Health Sciences Computer Facility and its staff. An IBM 2780 teleprocessing terminal gives access, over phone lines, to the IBM 360/91 computer at the UCLA Health Sciences Computer Center. Computer service is on a daily, batch job basis with turnaround within 12 hours. The terminal consists of a high speed card reader and punch and a 300-line per minute printer. All programs of the UCLA Biomed series are available as are excellent machine file storage service and Fortran and PL 1 programming support.

Clinical Facilities: The City of Hope Medical Center includes a 200-bed hospital and an outpatient department located on a 90-acre site 25 miles east of Los Angeles. The annual budget of the institution exceeds \$16 million, with partial support of basic and clinical investigation programs derived from research grants totalling over \$2 million annually. Clinical programs concentrate on the care of adult and pediatric patients with serious medical and surgical diseases of the lungs, heart, blood and the metabolic and endocrine apparatus. The Medical Center also has clinical services for cancer surgery, neurology and neurosurgery (especially pain problems) and medical genetics. Patients are accepted for diagnostic evaluation and therapy on referral from private physicians. Medical care is given at no cost to the patient; these costs are supported by a private, national fund raising organization. The medical staff numbers over 60 full time specialists; most hold faculty appointments at local medical schools. All necessary support services, including clinical and anatomic pathology and diagnostic and therapeutic radiology, are represented by the full time staff. A consulting staff of specialists from the local medical community augments the full time staff as consultants in fields not represented on the staff.

The Respiratory Disease Department is one of five units in the Division of Medicine. It is presently staffed by five internists who direct the care on a 36-bed inpatient service and attend in an outpatient clinic one morning per week. Patients represent the entire spectrum of chest diseases, which includes acute and chronic obstructive lung disease, lung cancer, pulmonary fibrosis, tuberculosis, other acute and chronic infectious diseases and various diagnostic problems. Programs of special interest, such as one to manage young adults with cystic fibrosis, have been established in conjunction with clinical research projects. Inpatient care is facilitated by the geographical grouping of all patients, staff offices and support services. The staff of the inpatient service includes a full time social worker, a pharmacist (who acts as a consultant and administers a unit dose drug system) occupational and physical therapists and a clinical nurse specialist. This nurse is trained in physical medicine in addition to nursing and serves as a resource person for the nursing staff and patients (in- and outpatients). The nursing staff is large and stable. An inhalation therapy service, administered by the Respiratory Disease Department, supplies the therapy needs of the chest patients and the rest of the hospital.

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The other services within the Division of Medicine are the Departments of Cardiology, Hematology, Medical Genetics and Metabolic Diseases. Although not directly involved in this program, their fourteen staff members supply necessary consultative and collaborative support and supervise specialized facilities such as the Cardiac Catheterization Laboratory, Electrocardiography and Vectorcardiography Laboratory, and Genetics Counselling Service. These medical departments also have active outpatient services, manage approximately 60 inpatient beds, and have their own programs of clinical investigation. Research programs in medical genetics are of special significance to the project outlined here. Active consultation is available on an ongoing basis from Drs. Ernest Beutler of the Hematology Department and David Comings of the Medical Genetics Department on problems of population genetics and molecular biology.

The Division of Radiology includes a Therapy and a Diagnostic Department and has a staff of eight physicians. Diagnostic equipment available includes two fluoroscopic machines with closed circuit television monitors and image intensifiers. These are equipped with 16 and 35mm cine and kinescope, as well as videotape recording capability. Two conventional linear systems for body section radiography and one system for transverse layerographs are available. Three rooms are equipped for chest, general radiography, and xeroradiography. The surgical suite is equipped with three x-ray installations; one is a complete catheterization lab facility and has 35mm cine along with TV monitoring of the image intensifier. Rapid film changing devices include a bi-plane Elema, a single plane Sanchez-Perez and a single plane Elema changer. The therapy service handles approximately 50 patients at any one time and its equipment includes a Theratron Cobalt 60 unit. In addition, equipment in the radioisotope diagnostic laboratory includes a scintillation camera, 5-inch crystal Picker Magna Scanner and Picker Dual Probe Ratemeter.

Other clinical divisions include Anesthesiology, Surgery and Neurology. The Anesthesiology Service is composed of four physicians, Cardiovascular Surgery includes a Thoracic Surgery Service with two senior staff members and Oncological Surgery with three. Surgical residents, rotated from local medical schools, augment the staff of the latter services. The Neurology and Neurosurgery staff serve as consultants on problems of pain and in other specialized areas. These services direct approximately 60 inpatient beds and also have active outpatient services. The Pediatrics Department includes a 20-bed inpatient service and an outpatient clinic devoted largely to the care of children with hematologic and solid tumors. Pediatric pulmonary problems of special interest are periodically accepted and cared for jointly with the Respiratory Disease Department. Cardiac, solid tumor, and other types of pediatric patients are handled in a similar joint fashion. The Pathology Division with seven staff members includes Departments of Clinical and Anatomic Pathology and of Cytology and Cytogenetics. Facilities include automated chemistry equipment and light and electron microscopes.

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In addition to these inpatient and outpatient facilities, the Medical Center has housing on its grounds for ambulatory care patients. Office, space, business equipment and furniture and other support facilities necessary for the conduct of the projects described here are readily available.

Other Support Facilities: Other support services, such as a Medical Library and an Electronics Instrumentation Repair and Development Laboratory, are available on the Medical Center grounds.

Facilities for Studies to be Performed at the Tel Aviv University: During his sabbatical leave the principal investigator will be affiliated with the Chaim Sheba Medical Center, a large government hospital which serves as a teaching unit of the School of Medicine, Tel Aviv University. Facilities there include an active inpatient and outpatient chest service and a pulmonary function laboratory equipped to perform routine studies as well as to measure diffusing capacity, lung compliance, work of breathing and arterial blood gases. The institution has agreed to make these facilities available part-time for studies as outlined in this proposal. Space is also available to carry out the antitrypsin assays outlined.

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11. BIOGRAPHICAL SKETCH: CHARLES MITTMAN

Title: Director, Respiratory Disease Department

Birthdate: R

Place of Birth: Chicago, Illinois

Education: A.B. University of Chicago, R
B.S. " "
M.D. " "

Honors: Alpha Omega Alpha; Sigma Xi; A.B., B.S., M.D. with honors

Major Research Interest: Pulmonary Physiology

Research and/or Professional Experience:

Director, Respiratory Disease Department, City of Hope
Medical Center, Duarte, California, 1966-present

Associate Clinical Professor of Medicine, University of
California at Los Angeles, 1973-present

Assistant Clinical Professor of Medicine, University of
California at Los Angeles, 1967-73

Instructor, Department of Medicine, University of Chicago,
1/66-12/66

USPHS Post-doctoral Fellow, Department of Medicine,
University of Chicago, 7/65-6/66.

Assistant Resident in Medicine, University of Chicago Clinics,
7/64-6/65

Assistant in Medicine, Johns Hopkins University School of
Medicine, Baltimore, 7/63-6/64

Assistant Visiting Physician in Medicine, Baltimore City
Hospital, 2/62-6/64

Surgeon, USPHS, Gerontology Branch, National Heart Institute,
Baltimore City Hospital, 12/61-6/64

McCoy College of Johns Hopkins University, elective courses in
Mathematics 9/62-64.

University of Chicago, Committee on Mathematical Biology,
7/65-6/66

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PUBLICATIONS - CHARLES MITTMAN

1. Mittman C: Nonuniform pulmonary diffusing capacity measured by sequential CO uptake and washout. J Appl Physiol 23:131-138, 1967.
2. Mittman C, Lieberman J, Marasso F and Miranda A: Smoking and chronic obstructive lung disease in alpha₁-antitrypsin deficiency. Chest 60:214-221, 1971.
3. Mittman C, editor. Pulmonary Emphysema and Proteolysis. Academic Press NY, 1972.
4. Mittman C, Barbella TV and Lieberman J: Alpha₁-antitrypsin deficiency as an indicator of susceptibility to pulmonary disease. J Occup Med 15:33-38, 1973.
5. Mittman C, Barbella TV and Lieberman J: Alpha₁-antitrypsin deficiency and abnormal protease inhibitor phenotypes in patients with lung disease. Arch Environ Health. In press.

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11. BIOGRAPHICAL SKETCH: JACK LIEBERMAN

Title: Associate Director, Department of Respiratory Diseases

Birthdate: R

Place of Birth: Chicago, Illinois

Education: B.A., University of California at Los Angeles, R
M.D., University of Southern California, R

Major Research Interest: Pulmonary Biochemistry

Research and/or Professional Experience:

Associate Director, Respiratory Disease Department, City of Hope Medical Center, Duarte, California, 1968-present.

Associate Clinical Professor of Medicine, University of California at Irvine, 1971-present.

Associate Clinical Professor of Medicine, University of California at Los Angeles, 1968-71.

Section Chief, Internal Medicine, Veterans Administration Hospital, Long Beach, California, 1963-68.

Clinical Investigator, Veterans Administration Hospital, Long Beach, 1960-63.

Research Fellowship (Heart), Harbor General Hospital, Torrance, California, 1958-60.

Attending Staff, Harbor General Hospital, Torrance, 1960-present

Consultant, Memorial Hospital of Long Beach, 1968-present

Consultant, Veterans Administration Hospital, Long Beach, 1968-present.

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PUBLICATIONS - JACK LIEBERMAN

1. Lieberman J: Digestion of antitrypsin-deficient lung by leuko-proteases. Pulmonary Emphysema and Proteolysis, pp 189-203. C. Mittman, editor. Academic Press, NY and London, 1972.
2. Lieberman J and Kaneshiro W: Inhibition of leukocytic elastase from purulent sputum by α_1 -antitrypsin. J Lab & Clin Med 80:88-101, 1972.
3. Lieberman J, Mittman C and Gordon HW: α_1 -antitrypsin in the livers of patients with emphysema. Science 175:63-65, 1972.
4. Lieberman J and Mittman C: A new "double-ring" screening test for carriers of α_1 -antitrypsin variants. Amer Rev Resp Dis, in press.
5. Lieberman J: Heat lability of α_1 -antitrypsin variants. Chest, in press.

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BIOGRAPHICAL SKETCH: E. EUGENE PEDERSEN

Title: Consultant in Epidemiology

Birthdate: R

Place of Birth: Milwaukee, Wisconsin

Education: B.A. California State College, Long Beach, R
M.A. California State College, Long Beach, R
PhD. Claremont Graduate School and
University Center, R

Honors: Psi Chi, National Honorary Society in Psychology, 1960
Dean's List, California State College, Long Beach
(5 semesters) 1960-62

Major Research Interest: Biostatistics, research design,
population study & environmental stress effects

Research and/or Professional Experience:

Co-Investigator, USC/Rancho Los Amigos Hospital, Downey,
CA. Statistician and Epidemiologist 1970-present.
Co-Investigator, USC/Rancho Los Amigos Hospital, Downey,
CA. Programs developed to study Behavioral Effects of
Environmental Stressor Agents 1970-present.
Assistant Professor of Psychology, California State
University at Los Angeles 1970-present.
Lecturer, USC School of Education 1969-70.
Instructor, Pepperdine University, Research Design and
Statistics, 1967.
Research Psychologist, Vocational Services, Rancho Los
Amigos Hospital, Downey, CA 1967-70.
Research Associate, Rancho Los Amigos Hospital, Downey,
CA, Attending Staff Association 1965-66.
Graduate Assistant, California State College at Long
Beach, Experimental Psychology and Learning Labs 1964-65.
Research Assistant, Psychology Department, V A Hospital,
Long Beach, CA 1961-64.

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PUBLICATIONS - E. EUGENE PEDERSEN

1. Wendland LV, Forney RL, Pedersen EE and Coss JG: The use of automated instruction with severely physically handicapped students. J Natl Soc for Programmed Instruct 6:(10) 409, 1967.
2. Coss JG, Forney RL, Wendland LV, Pedersen EE: Effectiveness of automated visual programmed instruction with paraplegic and other severely handicapped students, Final Report. Project No. 5-0411., U. S. Office of Education, Dept. of Health, Education and Welfare, December 1966.
3. Wetmore C and Pedersen EE: Selection of students for orthotic-prosthetic education programs. Prosthetic Res.Bull, Fall, 1969,ed.
4. Pedersen EE, Breisacher P and Hackney JD: Rapid assessment of tests of pollutant effects in man. Institute of Environmental Sciences, 1971 Proceedings "Living in Our Environment," 277-281, 17th Annual Technical Meeting, Biltmore Hotel, April 26-31, 1971.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

*C. Mittman, MD, Principal Investigator 30%
 J. Lieberman, MD, Co-Investigator 20%
 ***E. Pedersen, PhD, Consultant in Epidemiology 5%

Technical

T. B. Barbela, Research Technician 20hr
 L. Gaidulis, Biochemistry Technician 20hr
 W. McClelland, Pulmonary Function Tech 20hr
 ***To be filled, research Tech in Israel 40hr

Sub-Total for A

B. Consumable supplies (by major categories)

Blood drawing and storage 1,000
 Reagents, antibodies 1,000
 Gases, recording paper, forms, etc. 1,000

Sub-Total for B 3,000

C. Other expenses (itemize)

Computer time 500
 Repair of equipment, maintenance 500
 Local travel for survey work 500
 Travel to scientific meetings 1,000
 ***Fee paid to subjects brought to
 Medical Center in lieu of their
 day's wages 4,500

Sub-Total for C 7,000

Running Total of A + B + C 28,137

D. Permanent equipment (itemize)

*****Field spirometer for survey work 800

*, **, ***, ****, ***** see notes on next page

Sub-Total for D 800

E. Indirect costs (15% of A+B+C)

E 4,671

Total request 36,608

15. Estimated future requirements

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	300	7,000	1,000	2,993	33,943
Year 3	None					

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14A. Notes on budget items

* During the period of this grant Dr. Mittman will be on sabbatical leave at the Chaim Sheba Medical Center, Tel Hashomer, Israel, a unit of the School of Medicine, Tel Aviv University. He will directly pursue selected aspects of this project described in the proposal and will remain in contact with the activities at the City of Hope through correspondence and several week-long visits scheduled during the leave period. The day-to-day supervision of activities at the City of Hope will be the responsibility of Dr. Lieberman, the co-investigator.

** Dr. Eugene Pedersen, Epidemiologist, Respiratory Research Unit, Rancho Los Amigos Hospital, University of Southern California, has been serving as a consultant to this project and will continue in this capacity. He spends one to two days each month at the City of Hope and is receiving $\$2$ per month, the customary fee at this institution. His biographical sketch is included.

*** These funds will be sufficient to hire a full time research technician in Israel to perform antitrypsin assays and carry out the pulmonary function studies outlined in the proposal.

**** During the current grant period Kaiser Steel employees who have come to the City of Hope for detailed studies have been transported at the expense of Kaiser and have received their usual wages for the day from the company. For the coming year the company has indicated that funds to cover these wages will not be available. We propose to compensate each man selected for studies at the Medical Center by paying them a fee of \$30, an arrangement that is satisfactory to management and the union representatives.

***** The field spirometer will be used for studying various subjects in Israel, as outlined in the proposal.

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Genetic and Environmental influences on lung disease	NIH 12833	30,301	9/1/73 to 8/31/74
Alpha ₁ -antitrypsin deficiency in pulmonary emphysema	NIH 13398	49,480	1/1/73 to 12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
NONE			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made"

Checks payable to

C of Hope Medical Center

Mailing address for checks

1500 East Duarte Road

Duarte, California 91010

Principal investigator

Typed Name Charles Mittman

Signature *Charles Mittman* Date 7/13/73Telephone 213 359-8111 771
Area Code Number Extension

Responsible officer of institution

Typed Name Elihu King

Title Assistant Administrator

Signature *Elihu King* DateTelephone 213 359-8111 709
Area Code Number Extension

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PULMONARY DISEASE QUESTIONNAIRE

This questionnaire deals with your health and that of your family. Please answer in PENCIL as completely and accurately as possible. There will be a later opportunity to view the answers with an interviewer so that any confusing questions can be clarified. The questions are of two types. Some questions require that the answer be PRINTED on the designated line. Others require that you CIRCLE the correct answer. For example, a man would answer

SEX:

(1) Male

2) Female

1. NAME _____

2. ADDRESS: STREET _____

CITY, STATE _____

ZIP _____ PHONE # _____

3. BIRTHDATE: MONTH _____ DATE _____ YEAR _____

4. SEX: A) MALE B) FEMALE

5. MARITAL: A) MARRIED NOW B) NEVER MARRIED C) SEPARATED

D) DIVORCED E) WIDOWED

6. BORN IN: A) USA B) EUROPE C) ASIA D) AFRICA

E) OTHER NORTH OR SOUTH AMERICA F) OTHER

6a. PLACES OF RESIDENCE (PLEASE LIST ALL CITIES WHERE YOU HAVE RESIDED AND YEARS OF

RESIDENCE: _____

7. HEIGHT _____ 8. WEIGHT _____

9. OCCUPATION: WHAT IS YOUR JOB? _____

HOW MANY YEARS ON THIS JOB? _____

HAVE YOU DONE OTHER TYPES OF WORK BEFORE? A) YES B) NO

IF YES, LIST THEM AND YEARS WORKED.

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DO OR DID ANY OF THESE JOBS OR YOUR HOBBIES INVOLVE (CIRCLE ALL THOSE THAT APPLY)

- A) BREATHING DUST B) BREATHING PAINTS OR FUMES C) WELDING
 D) FOUNDRY WORK E) MINING F) SOLVENTS G) PESTICIDES
 H) RADIOACTIVITY I) WORK THAT CAN PRODUCE DISEASE OF LUNGS
 J) EXPOSURE TO HAIRSPRAY OR OTHER AEROSOL SPRAYS

10. EDUCATION: HIGHEST GRADE A) NONE B) GRAMMAR SCHOOL
 C) HIGH SCHOOL D) COLLEGE E) GRADUATE SCHOOL

11a. DO YOU USUALLY COUGH FIRST THING IN THE MORNING IN THE BAD WEATHER? A) YES B) NO

b. DO YOU USUALLY COUGH AT OTHER TIMES DURING THE DAY OR NIGHT IN THE BAD WEATHER?

- A) YES B) NO

IF YES TO #11a OR 11b:

11c. DO YOU COUGH ON MOST DAYS FOR AS MUCH AS 3 MONTHS OF THE YEAR? A) YES B) NO

11d. FOR HOW MANY YEARS HAVE YOU HAD THIS COUGH?

- A) LESS THAN 2 YEARS B) 2-5 YEARS C) 5 OR MORE YEARS

12a. DO YOU USUALLY BRING UP PHLEGM, SPUTUM, OR MUCUS FROM YOUR CHEST FIRST THING IN THE MORNING IN THE BAD WEATHER? A) YES B) NO

12b. DO YOU USUALLY BRING UP PHLEGM, SPUTUM OR MUCUS FROM YOUR CHEST AT OTHER TIMES DURING THE DAY OR NIGHT IN THE BAD WEATHER? A) YES B) NO

IF YES TO 12a OR 12b:

12c. DO YOU BRING UP PHLEGM, SPUTUM OR MUCUS FROM YOUR CHEST ON MOST DAYS FOR AS MUCH AS 3 MONTHS OF THE YEAR? A) YES B) NO

12d. FOR HOW MANY YEARS HAVE YOU RAISED PHLEGM, SPUTUM OR MUCUS FROM YOUR CHEST?

- A) LESS THAN 2 YEARS B) 2-5 YEARS C) MORE THAN 5 YEARS

12e. DURING THE PAST THREE YEARS HAVE YOU HAD PERIODS LASTING AT LEAST 3 WEEKS WHEN YOU HAD MORE COUGH AND PHLEGM THAN USUAL? A) YES B) NO

12f. IF YES, DID YOU HAVE A FEVER? A) YES - HOW HIGH? _____ B) NO

12g. IF YES, DID YOU MISS ANY WORKING DAYS DUE TO THIS ILLNESS? A) YES B) NO

12h. IF YES, HOW MANY DAYS? _____

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13. DOES YOUR BREATHING EVER SOUND WHEEZY OR WHISTLING? A) YES B) NO
14. HAVE YOU EVER HAD ATTACKS OF SHORTNESS OF BREATH WITH WHEEZING? A) YES B) NO
15. ARE YOU TROUBLED BY SHORTNESS OF BREATH WHEN HURRYING ON LEVEL GROUND OR WALKING UP A SLIGHT HILL? A) YES B) NO
16. DO YOU GET SHORT OF BREATH WALKING WITH OTHER PEOPLE OF YOUR OWN AGE ON LEVEL GROUND? A) YES B) NO
17. DO YOU HAVE BREATHING DIFFICULTY NOT RELATED TO EXERCISE? A) YES B) NO
18. IF YES, WHEN? A) DON'T KNOW B) _____
19. DOES THE SMOG AFFECT YOUR BREATHING? A) YES B) NO
20. DURING THE PAST 3 YEARS, HOW MUCH TROUBLE HAVE YOU HAD WITH ILLNESSES SUCH AS CHEST COLDS, BRONCHITIS, OR PNEUMONIA?
 1 2 3 4 5
 none | | | | great deal
21. DURING THE PAST 3 YEARS, HOW OFTEN WERE YOU UNABLE TO DO YOUR USUAL ACTIVITIES BECAUSE OF ILLNESSES SUCH AS CHEST COLDS, BRONCHITIS, OR PNEUMONIA? A) ONE TIME B) 2-5 TIMES C) MORE THAN 5 TIMES
22. DO YOU THINK YOU HAVE EVER HAD ANY OF THESE CHEST DISORDERS - ASTHMA, ANY KIND OF BRONCHIAL TROUBLE, OR EMPHYSEMA? A) YES B) NO C) DON'T KNOW
23. HAS A DOCTOR EVER TOLD YOU THAT YOU HAD ASTHMA, SOME KIND OF BRONCHIAL TROUBLE, OR EMPHYSEMA? A) YES B) NO
24. HAS A DOCTOR EVER TOLD YOU THAT YOU HAVE OR HAD ANY OF THESE ILLNESSES? (CIRCLE ALL THAT APPLY) A) TUBERCULOSIS B) BRONCHIECTASIS C) BRONCHIOLITIS D) HAY FEVER E) CYSTIC FIBROSIS F) INDUSTRIAL LUNG DISEASE (LIKE SILICOSIS, ASBESTOSIS, DUST DISEASE, ETC.) G) COCCIDIOIDOMYCOSIS (COCCI OR VALLEY FEVER) H) HISTOPLASMOSIS I) ULCER (STOMACH OR DUODENAL) J) LIVER DISEASE (CIRRHOSIS, HEPATITIS) K) DIABETES L) HEART TROUBLE M) HIGH BLOOD PRESSURE
25. PLEASE SUPPLY NAMES AND ADDRESSES OF DOCTORS (OR HOSPITALS) VISITED FOR THESE ILLNESSES AND DATE OF VISIT. HAVE YOU HAD A CHEST X-RAY IN THE PAST?

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26. DO YOU NOW SMOKE CIGARETTES REGULARLY, OCCASIONALLY, OR NEVER? A) REGULARLY

B) OCCASIONALLY (USUALLY LESS THAN 1 EACH DAY) SKIP TO #27

C) NEVER - SKIP TO #27

IF YOU SMOKE REGULARLY NOW: (IF YOU DO NOT USUALLY SMOKE AT LEAST ONE CIGARETTE EACH DAY, SKIP TO #27)

a) DO YOU INHALE? 1) YES 2) NO

b) DO YOU SMOKE CIGARETTES WITH FILTERS OR WITHOUT FILTERS?

1) WITH FILTERS 2) WITHOUT FILTERS 3) BOTH WITH & WITHOUT FILTERS

c) HOW MANY CIGARETTES DO YOU USUALLY SMOKE EACH DAY AT THE PRESENT TIME?

NUMBER PER DAY

d) HOW OLD WERE YOU WHEN YOU BEGAN TO SMOKE CIGARETTES? AGE

e) WHAT IS THE USUAL NUMBER OF CIGARETTES YOU HAVE SMOKED PER DAY SINCE YOU BEGAN TO SMOKE? (PLEASE GIVE BEST ESTIMATE. ONE PACK CONTAINS 20 CIGARETTES.)

NUMBER PER DAY

f) DO YOU OR DID YOU INHALE CIGARETTES? 1) YES 2) NO

g) IF YES, HOW DEEPLY? 1) VERY DEEP 2) SOME 3) LITTLE

h) IF YES, HOW MUCH OF THE CIGARETTE DO YOU INHALE? 1) MOST

2) ABOUT HALF 3) LITTLE

SKIP TO #28. (ANSWER #27 ONLY IF YOU DO NOT SMOKE REGULARLY NOW.)

27. IF YOU DO NOT SMOKE CIGARETTES NOW, DID YOU EVER SMOKE THEM REGULARLY OR OCCASIONALLY?

A) REGULARLY B) OCCASIONALLY (USUALLY LESS THAN 1 PER DAY)-SKIP TO #28

C) NEVER SMOKED CIGARETTES - SKIP TO #28

IF YOU DO NOT SMOKE CIGARETTES REGULARLY NOW BUT USED TO SMOKE THEM: (IF YOU HAVE NEVER SMOKED 1 CIGARETTE OR MORE EACH DAY, SKIP TO #28)

a) WHAT WAS THE USUAL NUMBER OF CIGARETTES YOU SMOKED PER DAY?

b) DID YOU INHALE? 1) YES 2) NO

c) HOW OLD WERE YOU WHEN YOU BEGAN TO SMOKE CIGARETTES? AGE

d) HOW OLD WERE YOU WHEN YOU STOPPED SMOKING CIGARETTES REGULARLY? AGE

e) WERE YOU INFLUENCED TO STOP BECAUSE YOU HAD A COUGH, WHEEZING, OR SHORTNESS OF BREATH? 1) YES 2) NO

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28. DO YOU NOW SMOKE PIPES OR CIGARS REGULARLY, OCCASIONALLY, OR NEVER? A) REGULARLY

B) OCCASIONALLY (USUALLY LESS THAN 1 PER DAY) - SKIP TO #29

C) NEVER - SKIP TO #29

IF YOU SMOKE PIPES OR CIGARS REGULARLY NOW: (IF YOU DO NOT USUALLY SMOKE AT LEAST 1 CIGAR OR PIPEFUL EACH DAY, SKIP TO #29)

a) HOW MANY PIPEFULS OR CIGARS DO YOU USUALLY SMOKE EACH DAY? _____

b) HOW OLD WERE YOU WHEN YOU FIRST SMOKED PIPES OR CIGARS? _____ AGE

c) DO YOU USUALLY INHALE WHEN YOU SMOKE EITHER PIPES OR CIGARS? 1) YES

2) NO SKIP TO #30

(ANSWER #29 ONLY IF YOU DO NOT SMOKE PIPES OR CIGARS REGULARLY NOW)

29. IF YOU DO NOT SMOKE CIGARS OR PIPES NOW, DID YOU EVER SMOKE THEM REGULARLY OR

OCCASIONALLY? A) REGULARLY B) OCCASIONALLY (USUALLY LESS THAN 1 EACH DAY)

C) NEVER - SKIP TO #30.

a) HOW MANY PIPEFULS OR CIGARS DID YOU USUALLY SMOKE EACH DAY? _____

b) HOW OLD WERE YOU WHEN YOU FIRST SMOKED PIPES OR CIGARS? _____ AGE

c) HOW OLD WERE YOU WHEN YOU STOPPED SMOKING PIPES OR CIGARS? _____ AGE

d) DID YOU USUALLY INHALE WHEN YOU SMOKED EITHER PIPES OR CIGARS?

1) YES

2) NO

30. DO YOU DRINK ANY ALCOHOLIC BEVERAGES?

A) YES

B) NO

IF YES: a) HOW MANY GLASSES OF BEER PER WEEK? _____ GLASSES

b) HOW MANY GLASSES OF WINE PER WEEK? _____ GLASSES

c) HOW MUCH HARD LIQUOR PER WEEK? _____ SHOTS OR _____ PINTS

31. DID YOU EVER DRINK MORE HEAVILY THAN YOU DO NOW?

A) YES

B) NO

32. DO YOU NOW USE ANY MEDICINES OR DRUGS?

A) YES

B) NO

33. IF YES, LIST THEM (KIND OF MEDICINE AND DOSE)

BRONCHODILATOR _____

EXPECTORANT _____

CORTISONE _____

ANTIBIOTIC _____

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DIGITALIS _____

IPPB _____

BIRTH CONTROL PILL, THE PILL _____

ESTROGEN, FEMALE HORMONE _____

INTRAVENOUS DRUGS _____

OTHER (KIND & DOSE) _____

34. WHAT PART OF THE WORLD DID YOU OR YOUR PARENTS OR THEIR ANCESTORS COME FROM?

(CIRCLE ALL APPROPRIATE ANSWERS)

A) FAMILY LIVED IN 'MERICA AS FAR BACK AS KNOWN

B) AMERICAN INDIAN, ESKIMO

C) SCANDINAVIA

D) BRITISH ISLES

E) FRANCE, BELGIUM OR HOLLAND

F) SPAIN

G) ITALY, GREECE

H) MIDDLE EUROPE

I) RUSSIA

J) INDIA, CHINA, SOUTHEAST ASIA,
OR

K) NEAR AND MIDDLE EAST, INCLUDING JEWS

PACIFIC ISLES

L) NORTH AFRICA

M) MID AND SOUTH AFRICA

N) OTHER

C. RACIAL BACKGROUND?

A) WHITE

B) BLACK

C) BROWN

D) YELLOW

E) RED

F) OTHER

36. HOW MANY BROTHERS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

37. HOW MANY SISTERS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

38. HOW MANY SONS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

39. HOW MANY DAUGHTERS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

40. HOW MANY GRANDSONS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

41. HOW MANY GRANDDAUGHTERS DO YOU OR DID YOU HAVE? LIVING _____ DEAD _____

42. HOW MANY UNCLES ON YOUR FATHER'S SIDE? LIVING _____ DEAD _____

43. HOW MANY AUNTS ON YOUR FATHER'S SIDE? LIVING _____ DEAD _____

44. HOW MANY UNCLES ON YOUR MOTHER'S SIDE? LIVING _____ DEAD _____

45. HOW MANY AUNTS ON YOUR MOTHER'S SIDE? LIVING _____ DEAD _____

46. DO OR DID ANY OF YOUR BLOOD RELATIVES HAVE ANY OF THESE DISEASES?

A) ASTHMA

B) BRONCHITIS

C) EMPHYSEMA

D) TUBERCULOSIS

E) BRONCHIECTASIS

F) BRONCHIOLITIS

G) CYSTIC FIBROSIS

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H) HYALINE MEMBRANE DISEASE (RESPIRATORY DISTRESS OF THE NEWBORN)

I) INDUSTRIAL LUNG DISEASE

J) HAY FEVER

K) ULCER (STOMACH OR

L) LIVER DISEASE

M) DIABETES

DUODENAL

N) HEART TROUBLE

O) HIGH BLOOD PRESSURE

P) LUNG CANCER

Q) MONGOLISM, OR ANY CONGENITAL OR INHERITED DISEASE

47. IF YES, PLEASE LIST WHICH RELATIVE, WHAT DISEASE AND IF THEY ARE LIVING OR DEAD.

48. (FOR WOMAN) DID ANY OF YOUR PREGNANCIES END IN A MISCARRIAGE, STILL BIRTH, OR

ABORTION?

A) YES

B) NO

49. IF YES, HOW MANY? _____

1003539795

cm/dm

2/15/72

CHRONIC BRONCHITIS RISK IN COKE OVEN WORKERS: INFLUENCE OF INHERITED FACTORS. C. Mittman, H. A. Lewis* and J. Lieberman**, Respiratory Disease Department, City of Hope Medical Center, Duarte, and Kaiser Steel Company, Fontana, California.

To assess the role of inherited factors in the development of chronic bronchitis, a survey was conducted among 309 coke oven workers. Each man responded to a standardized questionnaire; serum samples were analyzed for Pi phenotype. Approximately 162 of men had symptoms of chronic bronchitis. Symptoms were more common in older men and those who smoked most, but the frequency of bronchitis in the overall group bore no relationship to the duration or type of work exposure. Pulmonary function studies were performed on 14 men with abnormal Pi phenotypes (7MZ, 7MS) and 15 men with a P1MM type but a family history of emphysema or bronchitis (the experimental group). Findings were compared with 29 control subjects matched by age, work and cigarette exposure history and ethnic background. Chronic bronchitis was present in 14 of the 29 experimental subjects (48%), but in only 6 (21%) of the controls (difference significant, $p < .05$, Chi square). Airways obstruction was present in 5 bronchitics (34%) in the experimental group, but in only 1 of the control bronchitics (17%). In the experimental group bronchitis was more common in those who had worked in the oven area over ten years (9 of 13 men) when compared with men with ten or less exposure years (5 of 16 men). These findings suggest that this job exposure is a hazard for men with an abnormal Pi phenotype or a family history of bronchitis or emphysema. Since these factors can be determined at the time of employment and job placement, such cases of lung disease may be preventable.

1003539796

June 20, 1973

MEMORANDUM

TO: Drs. Hockett and Nordsiek

FROM: W. U. Gardner

SUBJECT: Grant 841
Progress Report #1 (October 1, 1972 - May 31, 1973)
Charles Mittman, M.D., City of Hope Medical Center, Duarte,
California.
"Hereditary Susceptibility to Bronchitis-Emphysema."

The progress made is largely an increase in numbers of patients from the Kaiser Steel Mill at Fontana, California, who have been examined and the development of STIC (serum trypsin inhibitory capacity) as a more rapid method for determining emphysema susceptibility. This may be packaged and made widely available for screening. It gives 2% false positives and a questionable area between 0.8 and 1.2 where results are questionable. This may be due to double active substances -- more than one inhibitor. Coke oven workers are highest risk for emphysema: 48% COPD (9 of 13 > 10 years, and 5 of 16 < 10 years).

Continuation: Mittman is on leave and will be in Israel. He is applying for a full-time technician there. He states Samaritan Jews reportedly have a high incidence of emphysema but other Jewish and Arab groups do not. He plans to continue his epidemiological studies in Israel and also do the screening and pulmonary functional testing in collaboration with physicians there.

At Duarte he will continue the pulmonary testing and screening for enzyme inhibitors adding several hundred more observations. He has a collaborating epidemiologist. From the report and continuation I do not feel too secure about the genetics and epidemiology. He has stated that there are other "familial" emphysema, possibly genetic, determiners of the disease than alpha-1 antitrypsin but, other than a "double ring," has no proof. He proposes to do studies on other steel mills but this is again vague. He proposes a vertical study at Duarte to assess predisposition to COPD and job assignments. He is making comprehensive pulmonary function tests on all. I would like to see more detail of Dr. Lieberman's input. I will be at Duarte in July and may learn more about the program and plans.

W.U.G.

1003539797

WUG:ek

June 28, 1973

MEMORANDUM

TO: Drs. Gardner and Nordsiek

FROM: R. C. Hockett

SUBJECT: Grant #841 Hereditary Susceptibility to Bronchitis-Emphysema
Visit by Charles Mittman, M.D., City of Hope Medical Center,
Duarte, California on June 27, 1973.
(See W.U.G. memo of June 20)

Dr. Mittman visited CTR office for about four hours in the forenoon while in town to make a presentation of his work at the AMA meeting.

High points of the discussion:

1. Data analysis. This is actively under way under E. Eugene Pedersen, Consultant in Epidemiology. They are booked into a large computer at U.C.L.A. Data are virtually complete on the coke oven workers who represent the highest risk group with respect to environmental exposures. Data include questionnaires, blood assays and clinical lung function measurements. Cooperation has been almost 100%.

Mittman showed trial runs, chiefly regression analysis. Important related factors are level of exposure, duration, age at start of exposure, smoking levels, age smoking started, phenotype, race.

Black people, of whom there are a good number, show low incidence of chronic pulmonary disease (as has been shown elsewhere). "Spanish" (Mexicans) are intermediate, about 1/3 the white rate. Orientals are very few in this sample. More definitive data will be obtained on phenotype etc. as related to race.

2. Supplementary progress report. Mittman agreed to submit an additional progress report about the end of August to include data and perhaps two manuscripts (1) coke-oven workers, questionnaire and phenotype data, (2) lung function data. The draft of a continuation application is to be formalized without substantial change.
3. Other groups under study. Foundry men now under study have a lesser exposure to air pollutants. Crane operators and workers in the tinning plant breathe essentially clean air.

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4. Familial susceptibility to COLD unrelated to anti-trypsin deficiency.

Evidence for the existence of such kindreds has emerged from the questionnaire data plus phenotyping. Mittman thinks that the Samaritans in Israel may prove to be such a group - reliably reported high incidence of COLD, long segregation and inter-marriage, geographical concentration. Arrangements have been made for STIC survey during his year in Israel. (Extent of complete phenotyping ?)

5. Levels of leukocyte proteases. In response to my question, Mittman expressed respect for Goldston's observations and conceded the importance of ratios between anti-trypsin activity and leukocyte protease levels as determinants of COLD susceptibility. But I gather that he does not plan to go into this now because the methodology is too laborious for mass surveys. We could press this point at a later date for a substudy on some selected group.

6. Other questions.

Q. Do their lung function studies distinguish between Chronic bronchitis and emphysema?

A. He thinks they do pretty well but of course there is no autopsy confirmation.

Q. Can they distinguish between acute and chronic effects of the industrial exposures?

A. They try mainly by looking up workers who have shifted to other environments and retired people. The latter are proving to be harder to locate than expected.

Q. Do people who are exposed to industrial air pollutants tend to smoke more than others (as suggested by certain other studies)?

A. This might be attacked through study of men who shift jobs to less polluted environments.

Q. Could data be obtained on age of onset of clinical COLD in relation to smoking, phenotype and industrial exposure?

A. At present, indications are obtained by ^{introducing} "duration of smoking" (age started) and "duration of job exposure" ^{data} into the computer.

Q. Though the rapid STIC test is useful for rapid screening of subjects to identify those with low functional anti-trypsin, will the complete phenotyping be carried out to complete the genetic picture of the subjects and their families?

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

-3-

A. Yes, insofar as possible.

Note: This should be followed up with Lieberman during the July visit.

Q. Will repeat lung function tests be made? (Forgot to ask.)

RCH:gh

1003539800

July 25, 1973

MEMORANDUM FOR THE FILE

SUBJECT: Grant 841
Charles Mittman, M.D.
"Hereditary Susceptibility to Bronchitis-Emphysema."
Site Visit: July 20, 1973.

Drs. Mittman and Lieberman had clinic in A.M. (regular Friday activity) and Dr. Peterson was in a smog tank with 3 other persons on a week exposure to experimental smog until 4:00 P.M., an E.P.A. program. The chest service has 40 beds with surgeons, radiologists and internists (pulmonary disease) using them. A full chest work-up is done on all surgical patients on all services.

Annual budget of City of Hope Medical Center is \$12,000,000, of which \$9,000,000 is hard, \$1,000,000 contracts and grants, and \$2,000,000 special gifts. In addition, for the next 3 years a \$10,000,000 per year building program will double bed size 200 to 400 beds as I recall. Most of the staff is on hard money except fellows. They have no resident training program but I assume that some fellows assume some resident responsibilities.

There is a large and very well-equipped clinical pulmonary lab in the main clinical building. The research unit where Dr. Lieberman has his office and lab is also very well equipped. Some CTR purchased but apparently many gifts from manufacturers. They have worked on the development of some equipment. Dr. Lieberman has a contact with Miles Laboratory to get some supplies in exchange for consultation. City of Hope seems very good at getting hands in pockets. Although it started as a TB sanitarium in 1913, it was not until a little over 20 years ago that the recently retired chairman and director really reorganized it, setting up 7 major units (as I recall) with pulmonary disease being one. Most patients I saw were elderly. It is a quiet and peaceful place. Almost all the staff who wished had time for research mostly supported (other than salaries of staff) by outside funds. Most staff had medical school appointments. Mittman's is at Irvine.

My major concerns with the program were (1) will it run with Mittman in Israel?, (2) will the Israeli program be any contribution?, (3) will the numbers of observations of the Fontana study be of any epidemiological value?, (4) how long will it be before the project wears out?, (5) what does Mittman mean by setting up other coke oven studies?, and (6) how much is known about the environment of the coke oven workers (particulates, gases, temperature, etc.)?

I did not go to Fontana. They apparently have a Kaiser health plan that requires annual physical and clinical examinations. Mittman has to assure the patients of the confidentiality of his studies, however, and some patients don't want the health officers to know of findings. Some findings have resulted in job changes.

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(1) The program will run with Mittman away. A questionnaire of the MRC type is set up for information on coke oven men, their families and relatives and the control groups. Most of the 300 coke oven workers have been studied. One hundred more coke oven workers are to be added to plant this year and will be added to study. Lieberman will do the Al AT and the phenotype determinations. The overall unit seems quite efficient. The technical staff able and enthusiastic. An engineer-programmer is interested in computer analyses. I asked if they planned a sample recheck on any coke oven workers. They thought it might be a good idea. As RCH mentioned, the correlations of a number of factors (a) year on job, (b) smoking history, and (c) family history showed significant correlations with COPD. Low AlAT was less significant. Lieberman was determining both M and Z levels in heterozygates. M may come back a time or two during year if needed.

(2) The Israel program is largely conjectural. Mittman has been in Israel at least twice. He has contacted two good pulmonary people in Israel. There are very few because this has been a low support area in Israel. He has received a few blood samples from some COPD patients in Israel but did not comment on results. The two doctors have the clinical know-how and he will assist in AlAT testing. There are 400-600 Samaritan Jews, some rural and some urban, and he thinks they will cooperate. He might get enough material on the Samaritan Jews and general population in one year but is quite sure he can set up a continuing epidemiological program at least. Smog is a problem in Israel and smoking rate is high. He had no idea of lung cancer incidence nor did I. I'll check Dr. Steinitz's report. He will hear about his leave in a week or two.

(3) Yes, I think the numbers will be adequate but there should be some control rechecks. (See RCH report.)

(4) It will take 1+ years - 1 1/4-1 1/2 - as estimated to get new coke oven workers, the 600-800 controls and 100 crane operators run and possibly more time for analyses. In two years at most the program should run out. The company health office is interested and helpful. He wrote up the program for an industrial health journal (J. Occup. Med. July 1973 Stokinger). Kaiser health officers now collect blood and they are picked up one time weekly. One technician can run 18/day. It looks to me as if this is a limited project and at least one year to get anything and maybe two years to complete. Mittman thought this was a reasonable program plan. He would have a new project or new projects to develop. An up-to-date report, possible preliminary publication, will be submitted before September 1.

(5) The new coke oven projects are independent studies. He knew Anna Baetscher when he was at Johns Hopkins and her successor Bernice Bohen. The latter is considering setting up a similar study with Bethlehem Steel. Mittman has been consulting and may be continuing. He intimated that Johns Hopkins might be looking for support.

(6) Not enough is known about environment and this should be encouraged. It's hot, men must wear insulated shoes to avoid blistering feet. I'm going to try to get more information on this. It seems to me that the management would want to do something on this aspect. People spending short daily periods in the coke oven areas seem as vulnerable as the longer daily exposure.

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Peterson thinks the data will be adequate for significance. Lieberman is following three cases of Hashimoto's disease and knows of one other with extensive emphysema. Some homozygous ZZ infant may die with pediatric liver disease. High antitrypsin in liver - not secreted may contribute to cirrhosis in these cases. Lieberman and Mittman are trying to follow these. They have 4 ZZ patients over 35 with no symptoms, 3 are women. Males seem more vulnerable. Blacks show about 1/4 as much low AlAT as whites, apparently in keeping with the 30% white genetic dilution.

Goldston's leucocyte protease may account for some familial clustering without low AlAT.

Ben Burrows, Prof. of Med., U. Arizona, Tuscon, has a very large population on COPD study. Following from childhood to old age, a cross section and longitudinal study. Would be good to check upon this. Apparently a SCORE grant from NIH.

Lieberman is working on breakdown products of AlAT. It can be broken into 6 fragments.

Lieberman and Mittman are following the 7th graders in a Junior High to determine incidence of asthma, allergies and influenza history. There may be a high flu, croup, etc., history in MZ.

AlAT binds not only in lung but in liver, adrenal and kidney. Tissues are now being fractionated to find binding component.

W.U.G.

WUG:ek

1003539803

#702C - NIDEN

1003539804

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

July 24, 1973

Grant Application No. 702C

To: The committee comprising Drs. Loosli, Sommers, and Wyatt

Subject: Albert H. Niden, M.D., Drew Postgraduate Medical School, Los Angeles
Continuation application No. 702C (no commitment)
"Effects of Cigarette Smoke, Noxious Fumes and Drugs on the Terminal Airways"

History

CTR Grant No. 702, with renewals and extensions, supported this study at Temple University, Philadelphia, from July 1, 1969 through September 30, 1972.

Grant No. 702A for 1972-1973, at Temple, was recommended by SAB for \$25,618 plus two additional years. When news came of Dr. Niden's probable move to the west coast, only interim amounts were awarded to keep his study viable pending the move.

When Dr. Niden was established at Drew Postgraduate Medical School last month, Grant No. 702B was awarded to him there to carry the study through December 31, 1973.

No. 702C requests \$25,618 plus two additional years. In view of the vicissitudes outlined above, the starting date for this request should be considered as January 1, 1974. As there is no commitment, this application competes as a new request.

Documents Submitted (attached)

1. Application dated April 12, 1973.
2. Brochure "Charles R. Drew Postgraduate Medical School" dated April 25, 1973.

Comments

Attached is a copy of Dr. Loosli's letter dated June 7, 1973, summarizing his site visit on that date.

Dr. Niden has been asked to provide a Progress Report no later than September 15, 1973. A copy will be sent to you as soon as received.

F.W.N.
F.W.N.

FWN:wg
Encls.

1003539805

Comm.

Dr. Loosli
Dr. Sommers
Dr. Wyatt

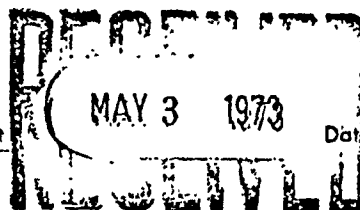
CHRONIC PULMONARY DISEASES

#762C

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., Inc.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8985

Application for Research Grant
(Use extra pages as needed)



Date: April 12, 1973

1. Principal Investigator (give title and degrees):
Albert H. Niden, M.D., Professor of Medicine, Director Pulmonary Disease Section
2. Institution & address: Drew Postgraduate Medical School
1620 East 119th Street
Los Angeles, California 90059
3. Department(s) where research will be done or collaboration provided:
Department of Medicine, Pulmonary Disease Section.
4. Short title of study:
Effects of Cigarette Smoke, Noxious Fumes and Drugs on the Terminal Airways
5. Proposed starting date: July 1, 1973
6. Estimated time to complete: Through June 30, 1976
7. Brief description of specific research aims:
 - a) To elucidate the role of the large alveolar cell, non-ciliated bronchiolar cell and monocyte-macrophage system in pulmonary phospholipid metabolism. Phospholipid metabolism will be studied by utilizing autoradiographic techniques to localize and follow the subcellular formation of phospholipids from fatty acid precursors. The effect of various stimuli e.g. cigarette smoke, noxious fumes and drugs on phospholipid metabolism by these cells will be observed.
 - b) To study pulmonary capillary permeability and the factors affecting it. By using E.M. tracer protein molecules of known sizes (horseradish peroxidase at 40 A°, hemoglobin at 65 A°, Dextran 40 A° to 200 A°) one is able to investigate the effects of intravascular pressure and damage to the capillary endothelium on pulmonary capillary permeability. The effects of air pollutants, drugs and cigarette smoke on pulmonary capillary permeability will thus be studied.

1003539806

8. Brief statement of working hypothesis:

- a) Three cell types in the terminal airways have now been shown to actively synthesize phospholipids--the large alveolar cell, the non-ciliated bronchiolar cell (Clara cell) and the monocyte-macrophage. Only from indirect evidence, the large alveolar cell is generally assumed to be the source of pulmonary surfactant. The role of the other cells--in particular--the Clara Cell in this process is not known. Finally, the effect of a marked increase in the monocyte-macrophages in the lung on the measured alterations in phospholipid metabolism (presumably surfactant) in pathologic states has not been considered by previous investigators. It is proposed that the Clara cell is intimately involved in surfactant production and marked changes in cell populations as readily observed in many pathologic conditions could effect phospholipid content and metabolism in the lung independent of alterations in pulmonary surfactant. The latter would lead to false conclusions by others as to presumed alterations in surfactant metabolism.
- b) The capillary endothelium appears to be sensitive to various noxious stimuli leading to an increase in pulmonary capillary permeability. Thus the common pathway for the various disorders leading to the Adult-Infant Respiratory Distress Syndrome ("Stiff Lung") is alveolar capillary endothelium damage with a transudation of fluid into the interstitium and alveoli.

9. Details of experimental design and procedures (append extra pages as necessary)

- a) Pulmonary Phospholipid Metabolism - The ultrastructural responses of the Clara cell, large alveolar cell, and monocyte-macrophages to various stimuli will be studied. Mice and hamsters will be exposed chronically to cigarette smoke (when Walton Horizontal Smoking machine becomes available - see Progress Report) or placed in a plastic, environmental chamber and exposed to 15% oxygen, 100% oxygen, 5% CO₂, nitrogen dioxide, etc. Tritiated fatty acids will be complexed with serum albumin and injected. Since 40 to 60% phospholipids are extracted by normal fixation -- embedding for E.M., present techniques will be improved to reduce the loss (see progress report). In addition, E.M. frozen tissue technique will be developed for E.M. lipid autoradiography. Standard techniques for light and E.M. autoradiography will be used to follow the synthesis of phospholipid in the lung at 2, 5, 10, 20, 60, 240 minutes after injection. (We have shown active incorporation occurs in the first few minutes following injection.) Using standard TLC techniques the tag will be localized among the lung lipids. Because of the rapid turnover of lung lipids, it is questionable that a pulse labelling occurs in vivo so that it might be difficult to sequentially follow phospholipid metabolism in the lung with such technique. Therefore, the in vivo studies will be compared with in vitro pulse labelling of lung tissue slices (see progress report). The autoradiographic-biochemical studies will be correlated with direct measurements of surfactant activity extract from experimental lungs as measured on surfactometer (Klaray-Greenfield).
- b) Pulmonary Capillary Permeability - Horseradish peroxidase (HRP Worthington) with a diameter of 40 Å, 0.5 mg in 0.05 ml saline, will be injected intravenously into tail vein of control and experimental mice. The mice will be sacrificed four minutes after injection. Tissue will be prepared for ultrastructural visualization of HRP by the Graham-Karnovsky method as well as for routine light and electron microscopy. Effects of exposure to cigarette smoke, 100% oxygen, 5% CO₂, as well as the injection of drugs (epinephrine, alloxan, ANTU, serotonin) on pulmonary capillary permeability will be studied. In later studies, it will be necessary to use larger animals in order to monitor pulmonary artery pressure to assess the relative role of direct effects on membrane permeability versus secondary effects from increased intravascular pressure. In all experiments, lung weight/body weight ratios will be used to assess degree of vascular congestion and/or edema. (This is insensitive to early changes in pulmonary capillary permeability (see Progress Report). In particular, we are interested in examining the reversibility and prevention of oxygen toxicity. Changes in pulmonary capillary permeability are an early sign of oxygen damage and appears to be a sensitive model for studying this problem (see Progress Report). Hemoglobin (65 Å) will be injected as a tracer of a different molecular size. Hemoglobin results in similar reaction product as HRP when incubated with DAB. Finally Dextran of varying known

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9. (continued)

sizes—40 Å, 75 Å, 120 Å, can be used as an electron dense probe for capillary permeability (see Progress Report). Hemoglobin and Dextran will be used to validate the use of Peroxidase.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Electron Microscopy - Histochemical - Biochemical laboratory, Drew Postgraduate Medical School under direction of principal investigator.

20' x 30'	General Lab	8' x 12'	Microtome
8' x 12'	E.M. Room	6' x 12'	Animal Exposure Room
8' x 12'	Dark Room		

Equipped with:

Hitachi HS 8 Electron Microscope	Durst S-45 EM enlarger
Porter-Blum MT-2 Microtome with Stereomicroscope	
3 Diamond knives	
General lab centrifuge	
Thelco Oven	
Vacuum Dessicator (Ladd)	
A-O Spencer Cycloptic Stereomicroscope	
Leitz Orthoplan-Orthomat photomicroscope	
Fully equipped Dark Room	
2 Environmental chambers (small animal)	
Brinkman TLC Sandwich Chamber	
Sorvall Tissue Chopper	
New Brunswick Environmental Water Bath Shaker	
Kinney Vacuum Evaporator	
Mettler Balance	
Corning pH Meter	

11. Additional facilities required:

LKB E.M. cryokit to cut frozen sections and eliminate loss of lipids without dehydration
Consumable supplies

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12. Biographical sketches of investigator(s) and other professional personnel (append):

Date of Birth: R Place of Birth: Philadelphia, Pennsylvania;
Education: University of Pennsylvania A.B. R, M.D. R

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See (12. append)

3a

12. (Continued)

Honors: Phi Beta Kappa 1949, Borden Award Undergraduate Medical Research

Intern, Hospital of the University of Pennsylvania	1953-1954
Instructor, Department Pharmacology (Drs. Carl Schmidt & Domingo	
Aviado) University of Pennsylvania	1954-1955
Resident in Medicine, University of Chicago	1955-1957
Instructor, Department of Medicine, University of Chicago	1957-1958
Assistant Professor of Medicine, University of Chicago	1958-1964
Visiting Professor of Medicine, University of Missouri	1963
Honorary Research Assistant (NSF Fellowship) Dept. Anatomy	
Kyushu University (Prof. E. Yamada) Fukuoka, Japan	1964-1965
Honorary Research Assistant, Institute of Pathology	
(Profs. H. Meesen and H. Schultz) Dusseldorf, Germany	1964
Associate Professor of Medicine, University of Chicago	1964-1968
Professor of Medicine, Temple University	1968-

Member:

REDACTED

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BIBLIOGRAPHY

ALBERT H. NIDEN

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13. Percutaneous Pleural Biopsy with a Curreting Needle: Special Reference to Biopsy without Effusion. A. H. Niden, B. Burrows, J. E. Kasik and W. R. Barclay. *Amer. Rev. Resp. Dis.* 84:37, 1961.
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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

Albert H. Niden, M.D.
Principal Investigator

40%

Technical

Graham, Clarence (Includes 17% Fringe Benefits)

100%

R

Sub-Total for A

R

B. Consumable supplies (by major categories)

Fixatives, Embedding medium, Chemicals,
Radioactive material
Photographic supplies
Small animals and care
Consumable supplies for E.M.
Glassware, plasticware

\$1,000

1,000

500

500

451

Sub-Total for B

\$3,451

C. Other expenses (itemize)

Travel to scientific meetings
50% Service contract for E.M. maintenance

300

900

Sub-Total for C

\$1,200

Running Total of A + B + C

\$18,972

D. Permanent equipment (itemize)

Diamond knife
LKB E.M. Cryokit

500

3,300

Sub-Total for D

\$3,800

E

2,846

E. Indirect costs (15% of A+B+C)

Total request

\$25,618

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	3,846	1,200	1,000	3,077	24,590
Year 3	---	4,451	1,200	1,000	3,312	26,391

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ok
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5.

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
PENDING OR PLANNED			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Ultrastructural Development of Human and Mouse Fetal Lung with Special Reference to Large Alveolar Cell	USPHS	\$36,755	July 1, 1973 to June 30, 1975

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made"

Checks payable to

Charles R. Drew Postgraduate
Medical School

Mailing address for checks

1621 E. 120th Street
Los Angeles, Calif. 90059

Principal investigator

Typed Name Albert H. Niden, M.D.Signature Albert H. Niden Date 4/13/73

Telephone

R

Area Code

Number

Extension

Responsible officer of institution

Typed Name Mitchell W. Spellman, M.D.Title DeanSignature Mitchell W. Spellman Date 5/18/73Telephone (213) 564-5911, Ext. 211

Area Code

Number

Extension

1003539815

CHARLES R. DREW POSTGRADUATE MEDICAL SCHOOL

1620 East 119th Street • Los Angeles, California 90059

Telephone: (213) 564-5911.

OFFICE OF THE DEAN

The Charles R. Drew Postgraduate Medical School is the educational partner of the Los Angeles County-Martin Luther King, Jr. General Hospital. It was founded in 1966 by the Drew Medical Society (Los Angeles Chapter of the National Medical Association) and the UCLA and USC Schools of Medicine. A 19-member Board of Directors governs the Drew School, with representation from the three founding groups, the immediate and Los Angeles communities and the national community.

The initial recruitment of faculty and assembly of the School were supported by a federal grant to the Watts-Willowbrook Regional Medical Program. This award was unique in RMP history. In making the award, the California Committee on Regional Medical Programs recognized that the health status of this community could not be improved without a total realignment of the resources. The Drew School, situated IN the community, can support these resources: teaching hospital, community-focused health care system and manpower development.

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RECEIVED APR 25 1973

The Drew School comprises 11 clinical departments. Our responsibilities are to provide patient care, graduate education of interns and residents, the continuing education of community health professionals, and the training of allied health professionals. A faculty of more than 60 full-time medical and dental specialists has been recruited as of April, 1973.

Of import nationally and locally is the responsibility of the Department of Community Medicine to stimulate and organize a rational health services system for the King Hospital service area. The development of such a model system, endorsed fully by the Los Angeles Board of Supervisors, will provide comprehensive health services that are adaptable to the needs of the nearly 500,000 residents here.

The people we serve are preponderantly black and have been deprived of most social and economic benefits. More than half the population is under age 25. Unemployment is appallingly high; public transportation and other services are constrained. By every measurement, the people we serve have had less opportunity for independence and growth than others in this region. Their health status reflects these inequities.

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The Drew School is committed to serve this citizenry with quality medical services -- in an environment where community participation is essential for genuine fulfillment. We have given high priority to establishing a Faculty of Allied Health Sciences, readying community residents for purposeful employment in a growing national industry. Similarly, we have pledged that the traditional tasks of medical schools -- education, patient care and research -- shall derive their substance from the primacy of community service.

Each Drew School department is affiliated with one but not both local medical schools (UCLA, USC). These affiliations assure concurrent appointments in the affiliating university, by which Drew School faculty retain the same prerogatives, privileges and responsibilities as other university faculty.

The first unit of King Hospital opened for patient care and educational training in March, 1972. The campus plan now includes an acute unit which could grow from 470 to 760 beds by 1976; a Community Mental Health Center-Mental Retardation Unit, ultimately comprising 148 beds; an intern-resident residence to accommodate more than 200 house staff; a Clinical Sciences Building for

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patient-oriented research activities; a Faculty of Allied Health Sciences building; a child care and development center; and facilities to house the faculty, basic research laboratories, library and other learning resources of the Drew School and the community.

Plans for this latter facility is one of the end-products of a Master Plan Study commissioned by the Drew School, which was conducted from May 1971 - April 1973. The Drew School is engaged in a master planning study to define institutional goals and objectives in light of community, regional and national commitments. The study is attempting to raise and answer critical questions concerning the School's relationships and destiny, including the possible growth into an undergraduate medical school.

Since July, 1971, the primary funding source for faculty is the Los Angeles County Department of Health Services, through a contractual arrangement patterned after existing County contracts between UCLA and USC and the affiliated County hospitals. Additional support from the County will be derived by participation in the training of allied health personnel. The School is securing additional support from Federal, State, and private sources. For example, the Drew School has received the first grant in California extending the MEDEX program to train independent duty military corpsmen as physician's assistants.

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Projects to assist foster home parents, to train psychiatrists for dealing with problems found in disadvantaged communities, to investigate the "lazy leukocyte syndrome," and many others have already begun.

The Drew School is pursuing health as a societal goal rather than treating disease as recurring crises. Our mandate is to engage the unwieldy tools of a medical center to uplift the spirit and life style of this community as part of its health aspirations.

The Drew School is adapting the traditional priorities of education, patient care, and research to serve foremost the needs of the community. This is not, we maintain, incompatible with the mission of the university and its obligations to society.

The brightest and most resourceful academicians are joining us to comprise a multiracial faculty. The nation, persons of all ethnic and cultural origins, should be able to discern something of themselves in the Drew School -- and to recognize it as an instrument of healing and reconciliation, a modality to advance and create knowledge, and a focus of national purpose.

1003539820

April 25, 1973

UNIVERSITY OF SOUTHERN CALIFORNIA
SCHOOL OF MEDICINE
2025 ZONAL AVENUE
LOS ANGELES, CALIFORNIA 90033

DEPARTMENT OF MEDICINE

June 7, 1973

Frederic W. Nordsiek, Ph.D.
Associate Scientific Director
The Council for Tobacco Research - USA
110 East 59th Street
New York, New York 10022

Dear Fred:

I visited the Martin Luther King Jr. General Hospital today. I met David Ulmer, M.D., Chief of Medicine, in who's department Dr. Niden will be. Dr. Fisher, his associate, took me about the hospital, including, particularly the pulmonary function laboratory and wards, and the electron microscopic laboratory in the Department of Pathology. Both are completely equipped with competent technicians on the job. Of course, the pulmonary function laboratory will serve only clinical patients. Being a new center, building continues at the Drew Postgraduate Medical Center. Laboratory animal research space is yet to be constructed. As a substitute, large air conditioned trailers are being made available for this purpose.

Dr. Ulmer is a highly personable individual, having obtained his M.D. degree from Washington University (St. Louis) '54. His post-graduate experience in one capacity or another was spent at the Massachusetts Institute of Technology and the Harvard Medical School of Boston. He was associate professor of Medicine at Harvard when he left to become Chief of Medicine (1972) at the Drew Postgraduate Medical School, University of California, the clinical facility being the Martin Luther King Jr. General Hospital.

This school affiliates both with the UCLA and USC Schools of Medicine. I believe those on the faculty of Medicine and Pediatrics hold appointments at USC. Those in Surgery and Obstetrics & Gynecology hold appointments at UCLA.

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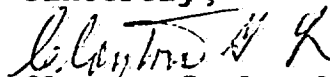
Frederic W. Nordsiek
June 7, 1973

-2-

Everyone seems to be highly pleased with the opportunities for teaching and research at the Drew Postgraduate Medical School. Dr. Ulmer looks forward to having Dr. Niden on the job.

I am sure Dr. Niden will have the full cooperation of the professional and technical staff and will be given the opportunity to carry out the studies outlined in his grant application.

Sincerely,



Clayton G. Loosli, M.D.
Hastings Professor of
Medicine and Pathology

CGL:ra

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#928 - RIFKIN

1003539823

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

August 1, 1973

Grant Application No. 928
PULMONARY

To: The committee comprising Drs. Jacobson, Loosli,
and Wyatt

Subject: Daniel B. Rifkin, Ph.D., The Rockefeller University
New application No. 928
"Proteases Produced by Mammalian Lung Tissue"

History

This proposal was Case No. 192 and formal application
was encouraged.

Application No. 928 requests \$36,077 plus two addi-
tional years.

Documents Submitted

Attached is application dated 7/17/73.

Copies of the publications listed under Item 13 of
the application have been provided and will be forwarded if you
so request.

FWN
F.W.N.

FWN:wg
Encl.

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Comm.

Dr. Jacobson
Dr. Loosli
Dr. Wyatt

PULMONARY

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET

NEW YORK, N. Y. 10022

(212) 421-8985

JUL 27 1973

Application for Research Grant

(Use extra pages as needed)

Date: 7/17/73

1. Principal Investigator (give title and degrees):

Daniel B. Rifkin, Assistant Professor of Chemical Biology, Ph.D.

2. Institution & address:

The Rockefeller University
New York, New York 10021

3. Department(s) where research will be done or collaboration provided:

Department of Chemical Biology

4. Short title of study:

Proteases Produced by Mammalian Lung Tissue

5. Proposed starting date:

January 1, 1974

6. Estimated time to complete:

Three years

7. Brief description of specific research aims:

The aim of this project is the characterization of a low molecular weight protease produced by human embryonic lung cells in vitro. This protease is a potent activator of serum proenzymes and may be involved in certain pathological conditions of the human lung, namely respiratory distress syndrome (RDS) and chronic emphysema. The proteases from human and mouse lung cells grown in culture will be purified and characterized as to their physical properties, mode of action, substrate specificity, and ability to activate proenzymes such as plasminogen procollagenase and pro-elastase. Antibodies will be made to the purified proteins. These antibodies will be used to determine at what stage in the embryonic development of the mammalian lung this enzyme appears and if it continues to be produced after parturition. Fluorescein labeled antibody will be employed as an immunofluorescent probe to test if other cells in preparations of fixed lung tissue are producing this enzyme. Finally, experiments will be designed to ascertain whether the production of the lung enzyme can be influenced by drugs such as corticosteroids.

It is hoped that this analysis will provide information necessary to determine if this protease may be involved in the respiratory disease, and, if so, how one may develop therapeutic approaches to these conditions.

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(see attached)

9. Details of experimental design and procedures (append extra pages as necessary)

(see attached)

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8.

We have recently reported that many neoplastic cells produce a proteolytic enzyme while their normal counterparts do not (1, 2). In the course of a study of transformed and normal human cells, it was found that normal human embryonic lung cells (fibroblasts) produce an enzyme which is similar in many of its characteristics to the enzyme produced by human tumor cells (3). This preliminary observation has been extended to cultures of human lung cells derived from four separate embryos (4). While the presence of proteolytic activities in lung are well known, they are usually attributed to leukocytes and macrophages (5-7). This is the first description of an extracellular protease produced by cells which are associated with the structural elements of the lung. It is our hypothesis that the control of production of this enzyme may be important in pathological conditions of the lung for the following reasons:

1) It has been reported that proteolytic treatment of rodent lungs produces a condition similar to that found in human chronic emphysema (8, 9). While it is not surprising that the rather harsh treatments utilized in these experiments caused tissue destruction, a source in human lungs capable of producing proteolytic activities in amounts adequate for such tissue destruction has yet to be described. The activities associated with leukocytes and macrophages are not substantial (10, 11) and may well be totally inhibited *in vivo* by serum inhibitors such as the α_1 -antitrypsin. However, the proteolytic activity generated by the lung fibroblasts is substantial. As few as 5×10^5 cells can produce enough protease to digest their own weight in protein in 17 hours even under conditions where serum inhibitors are present (11). It appears that the proteolytic inhibitors in serum do have an effect on the protease produced by lung fibroblasts but the rate of production of enzyme is great enough to overcome this inhibition. The α_1 -antitrypsin is the most effective of the serum inhibitors (12). This of interest because of the correlation of low levels of α_1 -antitrypsin and the propensity towards chronic emphysema in certain individuals (13-15). Finally, preliminary experiments suggest that this enzyme also can activate proenzymes such as procollagenase and proelastase. Our hypothesis is that either the abnormal production of the lung cell protease or the failure to inhibit the proteolytic activity which is normally generated by lung fibroblasts is responsible for chronic emphysema.

2) Respiratory distress syndrome (RDS) is correlated with a histological picture of hyaline membrane formation. Clinically it has been found that infants with RDS have low plasminogen (16) and low α_1 -antitrypsin (17-19) levels as well as smaller adrenal glands (20). We hypothesize that in infants with RDS there is an insufficient production of adrenal corticosteroids and therefore, a failure to inhibit the formation of the lung cell protease at birth, since it has recently been reported that corticosteroids prevent the production of proteolytic enzymes similar to the enzyme made by lung fibroblasts (21). Because of the continued production of the lung cell enzyme there is an abnormal activation of plasminogen. The plasmin thus produced is inactivated in turn by the circulating α_1 -antitrypsin. This combination of events would account for the clinical picture of plasminogen and α_1 -antitrypsin depletion. Moreover the lung would be unable to dissolve the hyaline membrane once formed since this structure is composed primarily of fibrin (22) and there would be little plasminogen available

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8. (continued)

for lysis. This type of hypothesis has been proposed before as an explanation of RDS (23) but the type of analysis used was extremely crude and, therefore, the results difficult to interpret in some cases. It seems worth while to reinvestigate the role of proteases, specifically the lung cell enzyme, in RDS.

With these facts in mind we propose to initiate an investigation of the role of the lung protease by first characterizing its physical and functional properties as well as the control of its synthesis using the experimental plan described.

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Initially our efforts will be directed towards the purification and characterization of the cell protease produced by both human and mouse embryonic lung cells in culture. Mouse cells will be prepared from trypsinized mouse embryonic lung, while the human cells will be isolated from the lungs of human fetuses obtained surgically. We have received assurances from physicians at Columbia-Presbyterian Hospital that it will be possible to secure the human tissue which we will need. Once these cells have been established in culture they will be checked for the production of the cell protease. This is done by incubating cells in medium lacking serum. The conditioned medium is removed, and clarified of debris by centrifugation. Aliquots of the supernatant solution are then incubated with purified plasminogen in plastic dishes which have been coated with ^{125}I -fibrin. The lung protease has been shown to activate plasminogen extremely effectively and the proteolytic activity of the plasmin formed is assessed by monitoring the release of ^{125}I from the surface of the dish. It should be noted that the activation of plasminogen in this system is accomplished by a proteolytic cleavage of the proenzyme and is, therefore, a true measure of the cell produced protease. (This assay has been published and is routinely used in our laboratory)¹.

The cell factor will then be purified from large quantities of conditioned medium. Preliminary experiments which we have performed indicate that the conditioned medium contains approximately 20 μg of enzyme per liter. Since the cells produce this enzyme continuously for several days in the absence of serum and the culture medium is saturated with active enzyme after 4-5 hours of incubation, large quantities of conditioned medium can be prepared with reasonable facility. (In a pilot experiment we have prepared 200 l of conditioned medium from human tumor cells in a three week period). The active enzyme will then be purified using the techniques of ammonium sulfate precipitation, SP-sephadex chromatography, gel chromatography, preparative acrylamide gel electrophoresis, and isoelectric focusing. These techniques have been used successfully to purify similar enzymes from transformed hamster and chicken cells (24, 25).

In this manner it should be possible to prepare milligram amounts of homogeneous enzyme. This material will then be used to characterize the physical and chemical properties of the enzyme. The molecular weight will be determined by SDS-polyacrylamide gel electrophoresis. Reactive groups in the molecule will be assayed using conventional techniques. For example, the requirement for disulfide bonds for an active enzyme will be tested by first reducing and alkylating any S-S-bonds and then testing for enzymatic activity. The type of protease will be assessed by testing proteolytic inhibitors of known activity such as DFP, TPLK, NPGb, E-ACA, TPCK (26). A number of naturally occurring inhibitors also will be screened since these may be particularly useful if this enzyme proves to be of any clinical significance. Experiments will be performed to see what molecules may act as substrates for the enzyme. A number of different substrates will be tested, such as BSA, casein, collagen, elastin, fibrin, and protamine. In addition the ability of this enzyme to activate proenzymes found in the serum or lung will be assessed. These will include proelastase, procollagenase, and prothrombin. These proenzymes will be obtained either from commercial sources or individual investigators and will be assayed by published procedures. We have determined that this enzyme can activate plasminogen extremely efficiently. Finally, attempts will be

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9. (continued)

made to determine if this protease has any activity upon the structural elements of the lung. Basement membrane from lung alveoli will be prepared as described by Kefalides (27), made radioactive by a labeling procedure (28), and the ability of the lung enzyme to degrade basement membrane assayed by the release of soluble radioactive material from the insoluble basement membrane.

The largest portion of the purified material will be used to produce antibodies in rabbits. (If we are unable to produce antibodies in rabbits, other animals will be used such as goats or sheep). A micro method will be employed where the antigen in an adjuvant solution is injected directly into a lymph node (29). This procedure is repeated several times at two week intervals. If successful it is possible to induce antibodies with 3-4 milligrams of purified material. This is undoubtedly the most crucial part of the research plan, since antibody against the lung protease will allow us to initiate some of the biological experiments described below. We are fortunate that there are several laboratories at Rockefeller which have had a great deal of experience in the production of antibodies to purified molecules and they have promised their assistance if necessary.

Having established the fundamental properties of the enzyme and having produced antibodies to the protease, we will then attempt to study those factors which may be important in the control of the synthesis of this protein. We will first attempt to follow the production of the enzyme histologically as a function of time using anti-lung protease antibody conjugated to a fluorescent dye (30). Mouse embryos and weanlings of various ages will be secured, and the lungs isolated, fixed, stained with the fluorescent antibody, and examined microscopically. This will allow us to determine at what time the enzyme is initially produced in the lung, for how long a period it is produced, and what other cell types besides fibroblasts produce it. Control tissue such as skin or muscle will also be tested since our own preliminary experiments indicate that fibroblasts derived from skin biopsies do not produce this enzyme. These experiments are extremely important because it may well be that cells which do not demonstrate an enzymatic activity when grown in vitro contain the enzyme in an inactive precursor form or in a form which is inhibited by a second molecule. It is hoped that these precursor or inactivated molecules will be detected by an immuno assay of histological specimens.

Experiments are planned to test the effect of drugs on the production of this enzyme. Lung cells will be grown in vitro in the presence or absence of different drugs and the amount of enzyme produced assayed either by its activity or by immune precipitation (31). The types of drugs which will be tested are adrenal steroids, immunosuppressors, polypeptide hormones, and drugs which affect platelet cell functions or fibrinolysis. If any of these drugs look promising in terms of either increasing or decreasing the production of the enzyme, experiments will be initiated to test the effects of these compounds in animals. The effects will be assayed immunologically on histological preparations.

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9. (continued)

Finally it is hoped that these tools will be applied to an analysis of the human lung in RDS and chronic lung emphysema. The main objective would be to study the occurrence and activity of this protein as a function of age, in relation to inhibitors and activators found in the lung, and the spatial distribution of enzyme within the tissue. This aspect of the work is at present only in the formative stage as it would require the collaboration of a number of physicians at neighboring hospitals and it is felt that it would be premature to set up such collaborations before more evidence is at hand as to the function of the lung enzyme.

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25. Acs, G. et al. Manuscript in preparation.
26. DFP, diisopropyl fluorophosphate; NPGB, nitrophenyl-p-guanidobenzoate; TLCK, N-d-Tosyl-L-lysyl-chloromethyl ketone; TPCK, N- α -Tosyl phenylalanyl chloromethyl ketone; E-ACA, ϵ -aminocaproate

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31. Fleissner, E. and Tress, E. *J. Virol.* 11: 250 (1973).

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The department of chemical biology contains all of the facilities necessary for the successful completion of this project. These include cold rooms, low temperature freezers, scintillation counters, tissue culture and media preparation facilities, incubators, spectrophotometers, etc. All of these facilities will be available for this study. Requests for equipment which would duplicate existing facilities have been made only for those items which are presently used to full capacity. The addition of those pieces of equipment requested will allow the proposed research to be carried out in an efficient and independent manner.

11. Additional facilities required:

The request for a biohazard containment hood is made because much of the proposed work will be with primary human tissue where the introduction of human pathogens into the laboratory is possible. At the present time the department does not have a containment hood for tissue culture work.

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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12.

Daniel B. Rifkin

Assistant Professor, The Rockefeller University

Born: REDACTED

Nationality: REDACTED

Education:

Princeton University	A.B.	REDACTED	Biology
The Rockefeller University	Ph.D.	REDACTED	Biochemistry
The Rockefeller University	Postdoctoral	REDACTED	Biochemistry

Honors:

Jane Coffin Childs Memorial Fund Fellow	1968-1970
American Cancer Society Faculty Research Awardee	1972-1974

Major Research Interests:

Biochemistry, cell transformation, cell biology, tissue culture

As a graduate student, I studied the protein and physical chemistry of several animal hemoglobins. This research was done under the supervision of Drs. William Konigsberg and Lyman Craig. Many of the techniques employed in the characterization of these proteins will be applicable to the study of the lung protease.

For the past five years I have studied the biochemistry of Rous sarcoma virus and the differences between normal and transformed cells. As a postdoctoral fellow in the laboratory of Dr. Edward Reich, I studied the lytic action of drugs which specifically kill transformed cells, the phospholipids of Rous sarcoma virus membranes as well as membranes from normal and transformed chick embryo cells, the effects of drugs which affect cellular RNA synthesis, and the organization of the proteins in Rous sarcoma virus. Since my appointment as assistant professor, I have developed a method for labeling proteins on the external membranes of vesicular structures. More recently I have been involved with the characterization of a novel proteolytic activity associated with malignant transformed cells. This research has given me the tissue culture and biochemical skills necessary for the successful completion of this project.

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12.

Lloyd Howard Waxman

Date of birth: REDACTED

Place: REDACTED

Marital status: REDACTED

Social security #:

Education:

Temple University	A.B.	Physics
(Summa cum laude)		
Temple University	M.A.	Physics
(NSF Traineeship in Physics)		
Harvard University	Ph.D.	Biophysics
(NIH Traineeship in Biophysics)		

Teaching Appointments:

Teaching fellow in Biology	1/69-6/69
Teaching fellow in Biochemistry	9/69-1/70
Teaching fellow in Biochemistry	9/70-1/71
Teaching fellow in Biochemistry	9/71-1/72

For the past four years I have been studying the oxygen-carrying proteins found in invertebrates. These proteins, which contain either copper or heme, generally have molecular weights in the millions, and are characterized by the high degree of cooperativity they exhibit upon binding oxygen. Consequently, they are of interest as models of protein-protein interaction over long distances, such as that which might be found in nerve conduction or on cell surfaces. The heme-containing pigments are composed of dimers which are held together by disulfide bonds and which contain one site for binding oxygen. The copper-containing proteins have polypeptide chains of molecular weight 3×10^5 , which places them among largest ever described. Each chain contains six sites for binding oxygen, and it appears that it has been duplicated in identical repeating units of 5×10^4 .

This project, which has some interesting genetic and evolutionary implications, has given me a great deal of experience in the purification and characterization of proteins and peptides, including a substantial amount of work with glycoproteins. More recently, because the main thrust in our laboratory has centered on membranes and membrane proteins, I have been using some of these techniques coupled with the use of detergents, to isolate various components of the erythrocyte and lobster nerve membrane.

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12. (continued)
L. Waxman

My present research interests are directed toward using techniques in protein, membrane, and carbohydrate chemistry to help characterize lesions in diseases which may involve the function of proteases or membrane proteins.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

Daniel B. Rifkin

50

Lloyd Waxman

100

REDACTED

Technical

John O'Malley

50

REDACTED

Staff Benefits

-

Sub-Total for A

B. Consumable supplies (by major categories)

tissue culture supplies

3,000

radioactive isotopes

2,000

chemicals

1,000

glassware

1,000

Sub-Total for B

7,000

C. Other expenses (itemize)

animal costs

500

publication costs

500

illustration costs

500

travel

500

Sub-Total for C

2,000

Running Total of A + B + C

26,328

D. Permanent equipment (itemize)

Baker Biohazard Containment

4,000

Microscope

1,500

Water bath

300

Sub-Total for D

5,800

E

3,949

Total request

36,077

E. Indirect costs (15% of A+B+C) (15% of 26,328)

15. Estimated future requirements: 5.5% increase 1 year

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	7,385	2,000	---	3,969	31,635
Year 3	R	7,791	2,000	1,000	4,170	34,247

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16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects

CURRENTLY ACTIVE			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Surface Proteins of Normal and Transformed Cells	NIH CA-13138	92,153	1/1/72-12/31/74

PENDING OR PLANNED			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Renewal of above grant in 1974			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Rockefeller University

Mailing address for checks

c/o Miss Margery Pedersen
~~Manager of Accounting Services~~
 The Rockefeller University
 New York, New York 10021

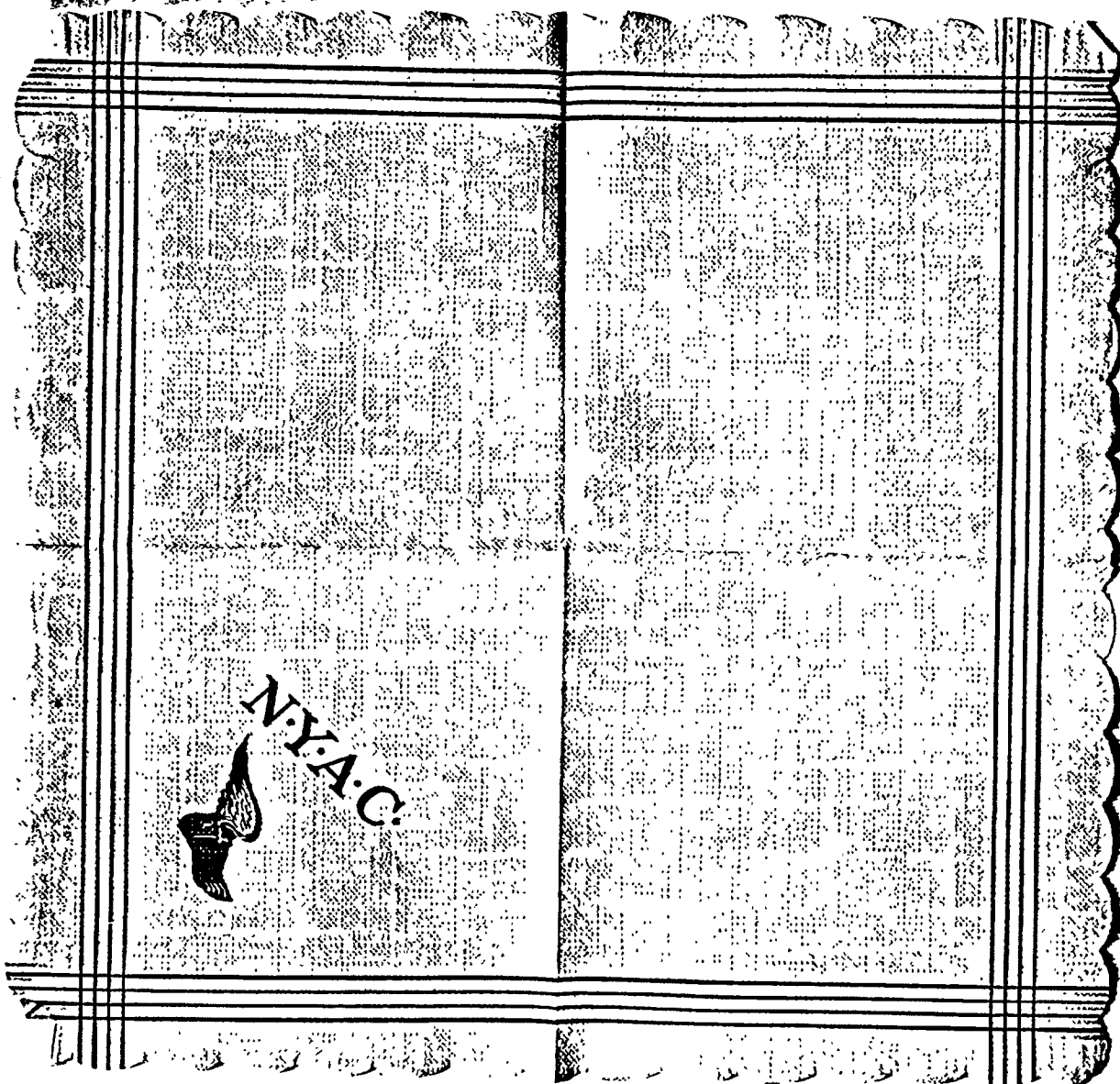
Principal investigator

Typed Name Daniel B. RifkinSignature Daniel B. Rifkin Date 7/17/73Telephone R
Area Code Number Extension

Responsible officer of institution

Typed Name Dr. Frederick SeitzTitle PresidentSignature F. Seitz Date 7/24/73Telephone R
Area Code Number Extension

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#913 - SBARRA

1003539841

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

June 5, 1973

Grant Application #913

To: The committee comprising Drs. Bing, Loosli and Wyatt

Subject: Anthony J. Sbarra, Ph.D.
New application No. 913
"The Interaction of Smoke, Alcoholism and Malnutrition on
Phagocytic Function"

History:

A preproposal was case #170 and application was encouraged.

Application #913 requests \$65,100 plus 2 additional years.

Documents Submitted (Attached)

1. Application dated May 25, 1973, with C.V.'s and publication lists of Drs. Sbarra, Selvaraj and Vitale.
2. Reprints of publications #77, #136, #147 and #152 in the bibliography of Dr. Sbarra are available here, and will be forwarded if you wish.

Comment

Dr. Lisanti has visited this applicant.

FWN:gh


F.W.N.

Enclosures

1003539842

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

JUN 1 1973

Date: May 25, 1973

1. Principal Investigator (give title and degrees):

Anthony J. Sbarra, Ph.D., Associate Director, Department of Pathology and Medical Research, St. Margaret's Hospital and Professor of Obstetrics and Gynecology (Microbiology), Tufts University School of Medicine, Boston, Mass.

2. Institution & address:

St. Margaret's Hospital
90 Cushing Avenue
Boston, Massachusetts 02125

3. Department(s) where research will be done or collaboration provided:

Pathology and Medical Research, St. Margaret's Hospital

Short title of study:

The interaction of smoke, alcoholism and malnutrition on phagocytic function

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

The studies to be carried out in this project will utilize alveolar macrophages and peripheral blood leukocytes isolated from smokers, alcoholics, undernourished patients, various combinations of these criteria and appropriate control subjects. The effect of *in vitro* addition of whole smoke, gas phase and water soluble phase, will also be investigated. Where pertinent, assays will also be conducted during or immediately following phagocytosis.

The experiments may be divided into three major categories: (1) energy production and uptake of particles; (2) metabolic activities involved in the production and utilization of microbicidal agents; and (3) microbicidal activities.

1. Metabolic activities involved in energy production and uptake of particles.

a. Glycolytic activity as measured by lactate production from both endogeneous substrates and added glucose; glycogen content of the phagocytes.

b. Tricarboxylic acid cycle activity as measured by $^{14}\text{CO}_2$ production from glucose-6- ^{14}C and succinate 1-4- ^{14}C ; respiratory activity of intact cells; oxidative phosphorylation by macrophage mitochondria as measured by P/O ratios with succinate and alpha-ketoglutarate as substrates.

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c. Lipid synthesis and turnover as measured by acetate-1-¹⁴C and ³²P incorporation in both whole cells and membrane fractions.

d. Phagocytic activity of intact cells with viable and heat killed bacteria and inert particles as measured by light microscopy.

2. Metabolic activities involved in microbicidal properties.

a. Hexose monophosphate shunt (HMS) activity as measured by glucose-1-¹⁴C and glucose-6-¹⁴C oxidation and glucose utilization; glucose-6-phosphate and 6-phosphogluconate dehydrogenase activities.

b. NADPH, NADH, L- and D-amino acid oxidase activities of phagocytes suspended in glycerin; H₂O₂ production and formate 1-¹⁴C oxidation.

c. Peroxidase activities of cell homogenates and subcellular fractions as measured by guaiacol oxidation, amino acid decarboxylation and iodination of bacteria; catalase activity of homogenates and subcellular fractions.

d. Lysosomal enzyme activities, i.e. phosphatases, proteases, nucleotidase, DNAase, RNAase, beta-glucuronidase and lysozyme.

3. Microbicidal activity.

a. Antimicrobial activities of intact phagocytes against gram positive and gram negative bacteria, viruses and fungi in presence and absence of serum.

b. Antimicrobial activities of subcellular fractions in correlation with peroxidase activities as measured by amino acid decarboxylation and iodination.

8. Brief abstract of working hypothesis:

This project is concerned with a systematic exploration of the total phagocytic process in alveolar macrophages and peripheral blood leukocytes collected from smokers, alcoholics and undernourished patients. Hopefully, the information derived from this study will establish what specific mechanism(s), if at all, are responsible for the increased susceptibility to infection commonly observed in these patients. Emphasis will be placed on the possible interaction of smoking, alcoholism and undernourishment on the overall host defense mechanisms of man. The study will include uptake of viable bacteria and inert particles, investigation of the various metabolic activities that are involved and influenced by the phagocytic process and a critical evaluation of the different bactericidal mechanisms that operate in the whole cell and different subcellular fractions.

Within the past decade considerable advances have been made in our knowledge and methodology of the total phagocytic activity of leukocytes. For example, this laboratory and others have demonstrated that particle uptake is an active process requiring glycolytic activity in polymorphonuclear neutrophils and oxidative energy in alveolar macrophages. However, inactivation of bacteria appears chiefly to be

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related to the phagocytosis associated oxidative events. For example, phagocytosis stimulates production of H_2O_2 and increases peroxidase activity. These reactants couple with a halide to form a potent antimicrobial system.

Knowledge such as this may now permit us to study systematically the total phagocytic process in the smokers with and without the additional stress of alcoholism and/or undernourishment from a vantage point heretofore not possible. Hopefully the knowledge to be gained from this investigation will have immediate practical application.

a. Smoking and host defense. It is generally held that smokers are especially susceptible to respiratory infections (1-5). However, contrary evidence has been frequently reported. For instance, Boake (6) found no differences due to smoking habits in respiratory illness in a study of 59 different families. Dowling et al (7) observed no statistical differences due to smoking in a double blind study where they exposed 328 non-smokers and 249 smokers to "infectious cold agent" and 111 non-smokers and 78 smokers to placebo. Brown and Campbell (8) reported that tuberculosis is associated more with drinking habits rather than with smoking habits. In a study of 100 patients, Potter et al (9) concluded that the normal bronchial clearance mechanism is able to prevent colonization of pathogenic bacteria and this ability is not impaired in smokers. Post-operative pulmonary complications were reported to be high in smokers (10); although Wilklander and Norlin (11) found no evidence to support such a hypothesis in 200 patients undergoing operations in winter months. Presumably other factors such as alcoholism and undernutrition rather than smoking are involved in the higher incidence of respiratory diseases in smokers. Controlled studies and specific in vitro examination of the recently described mechanisms of the phagocytes should be fruitful in the elucidation of the possible potentiating effects of other stress factors.

Many reports have appeared in the literature regarding the effect of smoke on the pulmonary clearance of both inert particles and viable bacteria. Necessarily most of these studies were in experimental animals and generally showed an impaired function in vitro. Studies on living animals are, however, equivocal. Albert et al (12) and Holma (13) found decreased clearance on acute smoke exposure, whereas LaBelle et al (14) found no ill effects of smoke on pulmonary clearance. Human tracheal and lung clearance in smokers is reported to be normal by Luchsinger et al (15); Albert et al (16) reported, however, that 8 out of 14 smokers demonstrated decreased clearance rates. Nutritional status and drinking habits of these subjects were not reported. In a study of life time elderly smokers and non-smokers, Paiva et al (17, 18) found that neither percent clearance nor half-time of clearance are altered due to smoking. Multiple regression analysis for different variables such as sex, age, height, smoking, forced expiratory volume vital capacity and flow rate indicated that only age alters clearance rates at a statistical significance of around 10%. Besides these conflicting reports, it should be emphasized that pulmonary clearance kinetics by itself may not be a good index of pulmonary defense capacity. Green and Kass (19) in an elegant study using ^{32}P labelled Staphylococcus aureus or

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Proteus mirabilis have demonstrated that pulmonary clearance of bacteria and bactericidal activity do not parallel each other. In vitro investigations with isolated phagocytes and with the newly available methodology may prove to be a fruitful approach in the resolving of these apparent paradoxes.

The effect of smoking on particle uptake and bactericidal activity of alveolar macrophages has received considerable attention in the past few decades. Most of these investigations indicate that alveolar macrophages isolated from either smokers or animals exposed to smoke show no alteration in their ability to ingest particles; phagocytic activity is also not altered on addition of smoke in vitro. However, bactericidal activity appears to be decreased under these conditions (20-24). Also, LaBelle et al (25,14) found that phagocytosis was not affected on exposing rats to cigarette smoke. It is well established now that particle uptake and bactericidal activity depend upon different metabolic activities of the phagocyte. Since smoke appears to decrease bactericidal activity without altering uptake, an investigation of the effect of smoke on the various metabolic activities of the phagocyte should increase our understanding of possible ill effects of smoke on pulmonary defense mechanisms. Further, a complete understanding of any ill effects of smoke may pave the way for possible corrective measures.

Smoking appears to have toxic effects on phagocytes other than alveolar macrophages. Thus, Eichel and Shahrik (26) have shown that the respiratory and glycolytic activities of oral leukocytes are considerably decreased following smoking. Since, at least some of the toxic agents in smoke are soluble in water (20,27) and nicotine alters the respiratory activity of alveolar macrophages (28), it is possible that the effects of smoke might be reflected by all the phagocytes of the host including the peripheral blood leukocytes. Since these cells are easily obtained and constitute a well defined model of one of the major host defense system, a systematic investigation of these systems will be of considerable interest.

Some investigators have examined the effects of smoke and its constituents on phagocytes at the molecular level. Thus, Kyle and Riesen (29) have shown that lung mitochondria loses oxidative phosphorylation efficiency on smoking. A general disruption of internal mitochondrial structure has also been reported (30). Nicotine has been demonstrated to decrease ATPase activity of sheep alveolar macrophages (28). Powell and Green (31) have reported that smoke decreases 3-phosphoglycerate dehydrogenase activity of rabbit alveolar macrophages but did not affect glucose-6-phosphate and lactate dehydrogenases. A decrease in oxido-reductases in biopsy material from smokers by histochemical techniques has also been reported (32). In spite of these numerous studies, proper emphasis on the metabolic and enzymatic activities of these phagocytes that are directly involved in particle uptake and microbicidal function is still lacking. Also, these investigations should be carried out when the phagocytes are actively involved in the performance of their physiological function, i.e. during engulfment, so that a proper understanding of the correlation of the metabolic activities and susceptibility to infection might result.

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b. Alcoholism and host defense. In most of the studies on the effect of smoke on the host defense mechanism, sufficient attention has not been paid to the possibility of other stress factors that might be acting independently or synergistically to decrease resistance to infection. There is some preliminary evidence in the literature to indicate that other stress factors may indeed play a major role in the increased susceptibility to infection commonly attributed to smoking. For instance, although smokers are generally considered to be more susceptible to tuberculosis, alcohol consumption has also been suspected as a factor in the etiology of tuberculosis; however, the evidence for the involvement of alcoholism has been inconclusive (33). As early as 1924, Stillman (34) reported the persistence of inspired bacteria in lungs of mice which received alcohol. In a more recent investigation of this problem, Brown and Campbell (8) have concluded that alcohol and not smoking was more directly associated with tuberculosis. Pulmonary clearance of viable bacteria has been shown to be decreased by ethanol intake (35). This effect was found to be dose related and was unrelated to ethanol induced respiratory depression. Alcohol has also been shown to have a migration inhibitory effect on alveolar macrophages (36), peritoneal leukocytes (37,38) and peripheral blood leukocytes (39). Phagocytosis is also reported to be decreased following alcohol intake (37,38).

The mechanism by which ethanol interferes with phagocytic function is not known. Numerous clinical reports of post alcoholic hypoglycemia have appeared in the literature. Also, liver biopsies of alcoholic patients have revealed that glycogen content is low or completely absent (40-43). In animals decreased hepatic glycogen levels after ethanol infusion even with normal blood glucose levels have also been reported (44). It is possible that leukocyte glycogen levels also decrease due to alcoholism. Since leukocytes derive their energy for phagocytosis from their glycogen stores and blood glucose, the possible malfunction of leukocytes in alcoholism might be due to a lack of energy production. Alcohol may also interfere in the functions of the phagocyte through the induction of the microsomal ethanol oxidizing system (45). NADPH oxidase is a vital component in the microbicidal system providing H_2O_2 for the peroxidase- H_2O_2 -halide system. Since the microsomal ethanol oxidizing system competes with NADPH oxidase for NADPH, low H_2O_2 production leading to decreased bactericidal activity might result. It is obvious from the above that the possible interaction of alcoholism with smoking needs careful investigation.

c. Malnutrition and host defense. Another possible stress factor that affects host defense mechanisms and may play a major role in the increased susceptibility to infection observed in smokers is malnutrition. Green and Kass (35) have demonstrated that acute starvation decreases pulmonary clearance significantly. Protein deficiency is known to decrease phagocytic activity of blood leukocytes (46) and peritoneal macrophages (47). Morbidity rates in iron deficiency (48) and urinary tract infections in anemia (49) are reported higher than in normal. Both thiamine and riboflavin deficiencies in rats and mice result in increased fatality rates following infection with Diplococcus pneumoniae (50-52). Folic acid deficient guinea pigs are also more susceptible to infections (53). Respiratory, genito-urinary and other

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infections are reported by many workers to be more frequent, persistent and often fatal in vitamin A deficient subjects (54-59). Leukocytes from animals fed diets that are low in protein and vitamins exhibit depressed phagocytic activity (60). Recently we have demonstrated a markedly decreased bactericidal activity and a considerably low phagocytic stimulation of hexose monophosphate shunt activity in leukocytes isolated from children suffering from severe malnutrition (61). The major biochemical defect in these phagocytes appears to be a low NADPH oxidase which also fails to respond with the normal stimulation observed in control subjects during phagocytosis (62). Since smoking suppresses appetite and smokers generally have a history of poor dietary habits, the contribution of the nutritional status of the smoker to the reported increased susceptibility to infection may be pertinent.

The interaction between smoking, alcoholism and undernutrition is complex. Alcoholics are generally undernourished because of their altered pattern of food intake caused by the high calorie contribution of alcohol intake without providing the essential nutrients. Also, recent evidences indicate that ethanol decreases the absorption of essential nutrients such as folic acid (63), thiamine (64), vitamin B₁₂ (65) and amino acids (66-68). In addition, alcohol may also increase the requirements of vital nutrients; for instance, extra folate is reported to be required by the alcoholic for normal bone marrow function (69). Thus, alcoholism by direct and indirect mechanisms aggravates undernutrition. Undernutrition also interferes with alcohol metabolism causing a vicious cycle to operate. For instance, half-life of blood alcohol after ingestion in rats is markedly prolonged in protein deficiency. Since both undernutrition and alcoholism adversely affect host defense mechanisms and a high percent of the smoking population is also alcoholic and hence undernourished, the general concept that smokers are more susceptible to infection needs a critical re-evaluation.

9. Details of experimental design and procedures.

a. Description of groups to be studied.

(1). Smokers, ex-smokers and non-smokers: Our patient population consisting of smokers will be derived mainly from the Veterans Administration and Boston City Hospitals in Boston. The degree of smoking will be evaluated by questionnaire method. It is anticipated that an ample supply of patients will be available. Groups will be made from 8-10 patients undergoing bronchoscopy per week. It is expected that some of these patients will have pulmonary infection. Where possible these patients will be examined before, during and after treatment.

(2). Alcoholics: Chronic alcoholics will consist of patients recuperating at Boston City and Veterans Administration Hospitals with and without cirrhosis of the liver and the following: iron deficiency anemia; folate deficiency anemia; iron and folate deficiency anemia. Patients with any superimposed infections will also be studied. Acute alcoholics (recent drinking) with and without infections will be

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patients who arrive at the Alcoholic Clinic of Boston City and Veterans Administration Hospitals inebriated within the last 24 hours; they usually are admitted in a state of intoxication. They are automatically hospitalized for nine days, after which they may be discharged or admitted to the medical ward for further study, if further medical attention is indicated. These patterns are referred to the Clinic by social agencies. There is no limit as to the number of alcoholic patients available. All patients will be studied within one week of their admission and studies repeated prior to discharge (except for cirrhosis, patients are discharged well and without anemia). Appropriate and comparable (i.e. sex, age, socio-economic status) controls will be similarly studied.

(3). Undernourished: Undernourished subjects for this study will be derived mainly from the out-patient clinics of St. Margaret's Hospital, Boston, Veterans Administration Hospital, Boston and Boston City Hospital, Boston. Pregnant women with anemia which is primarily nutritional in origin are readily available at St. Margaret's Hospital for study. Patients who appear to be undernourished and come to the outpatient clinics of Veterans Administration and Boston City Hospitals with minor illnesses will be referred to the laboratory. Social agencies will also be referring undernourished people to these hospitals. Some of these subjects will also be smokers. Phagocytes will be isolated from these subjects for study and where possible they will be followed after rehabilitation.

Approximately 8 to 10 patients per week are bronchoscoped and will be available for this study. Dr. Joseph Vitale will coordinate in this aspect. It is expected that this population of patients will permit us to develop groups as indicated in the table below. Both alveolar macrophages and peripheral leukocytes when desired will be obtained.

The classification of the various groups to be studied is shown in the table below.

GROUPS OF SUBJECTS FOR INVESTIGATION

Group	Smoking	Poor nutritional status	Alcoholism
1	-	-	-
2	-	-	+
3	-	+	-
4	-	+	+
5	+	-	-
6	+	-	+
7	+	+	-
8	+	+	+

- absent
+ present

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b. Evaluation of nutritional status. On each group the following, where appropriate, will be determined using conventional or micro techniques. Anthropometric measurements, especially height, weight and skin fold thickness, will be obtained and related to normal values recommended by the World Health Organization. A dietary survey by questionnaire method will be conducted to assess the history of previous food intake. Where feasible (especially in hospitalized subjects), food intake will be measured to evaluate the effect of treatment. Biochemical assessment will be used to specify the nutritional deficiency encountered. Blood analysis will include hemoglobin, hematocrit, peripheral blood differentials and PMN lobe counts. Serum analysis will be used to estimate iron, total iron binding capacity, folate (both N5-methyl-tetrahydrofolate and tetrahydrofolate), vitamin A, proteins by electrophoresis and pertinent serum enzymes. Alcohol acetalddehydes and triglycerides will also be estimated in alcoholic subjects. Erythrocyte transketolase and GSSG reductase will be estimated to assess thiamine and riboflavin nutritional status. Urine will be examined for albumin and bacteriuria. Standard liver function tests will also be conducted and where possible light and electron microscopic evaluation of liver morphology will be done from biopsy material.

c. Evaluation of host defense mechanisms.

(1). Isolation of phagocytes: Leukocytes will be isolated aseptically from peripheral blood by the dextran flotation technique (70). Blood will be drawn into a syringe containing 0.5 ml of 20% dextran and 200 units of heparin per 10 ml of blood. The blood, heparin and dextran suspensions will be mixed, all bubbles expelled and the syringe permitted to stand in an inverted position at room temperature for 30 to 45 minutes. The resulting buffy coat leukocytes will be expelled through a bent needle and washed twice in buffer medium, centrifuged at 200 g for 5 minutes and made up to the desired volume with buffered medium. Total and differential leukocyte counts will be made from both the peripheral blood and isolated suspensions by conventional techniques. The resulting cells will be used in experiments described below.

Alveolar macrophages will be isolated essentially by the method of Finley et al (71). Briefly, using topical xylocaine anesthesia, the trachea will be intubated with a size 19 Metras bronchographic catheter under fluoroscopic control. By inflating the later cuff at the catheter tip, a portion of the lower lobe will be isolated and lavaged with four to five 50 ml aliquots of normal saline. The lavage fluid will be centrifuged twice at 250 g for 10 minutes. The isolated macrophages will be brought up to the desired concentration and used for the different studies.

(2). Cell homogenate preparation: Cells to be homogenized are suspended in 0.25 M sucrose. They are homogenized for 2 minutes at 3800 rpm in a glass Potter-Elvehjem homogenizer with a motor driven teflon tipped pestle. Homogenization and centrifugation is done at 4°C in a Servall RC-2 centrifuge. The uncentrifuged homogenate, the 20,000 g 30 minute pellet fraction (lysosomes) and the 20,000 g 30 minute supernatant fluid fraction are to be studied for bactericidal and metabolic activities.

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Mitochondria from alveolar macrophages will be prepared by conventional techniques. Macrophages are homogenized in 0.25 M sucrose containing 0.07 M mannitol and 0.02 M ethylenediaminetetra acetic acid (pH 7.4) and centrifuged at 600 g for 10 minutes to remove nuclei and unbroken cells. The pellet is rehomogenized and recentrifuged at 600 g for 10 minutes. The combined supernatants are then centrifuged twice at 17,000 g for 20 minutes. The mitochondrial pellet is resuspended in 0.25 M sucrose containing 0.07 M mannitol and 0.1 mM MgCl_2 and 0.01 M phosphate buffer, pH 7.4

(3). Effect of smoke in vitro: Smoke for in vitro studies will be drawn from both filter and non-filter types of cigarettes in a 50 ml syringe modified to accept a cigarette. A measured volume of the smoke from the second syringe-fill, either before or after filtration through buffer at pH 7.4, will be introduced into the reaction vessel covered with a rubber cap. When necessary the effect of water soluble agents will also be investigated.

(4). Procedures for studies of metabolic activities: Lactate estimations will be done by the colorimetric method of Barker and Sumerson (72). Glucose utilization will be measured by utilizing glucose oxidase (73). Glycogen will be estimated as glucose equivalents by glucose oxidase following hydrolysis. Radioactive CO_2 production from labelled substrates will be absorbed in 20% KOH contained in the center wells of Warburg flasks and counted in Bray's solution in a Packard Tri-carb liquid scintillation spectrophotometer. Oxygen consumption will be measured either in a Warburg apparatus or with an oxygen electrode. Oxidative phosphorylation at site 2 will be assayed by the method of Lee et al (74). For phosphorylation at site 3, succinate or alpha-ketoglutarate will be replaced by 0.5 M ascorbate N, N, N, N' -tetramethylphenylenediamine (0.3 M) and 0.1 $\mu\text{g/ml}$ antimycin A_1 . Acetate ^{14}C and ^{32}P incorporation into whole cells and into membrane fractions will be studied as described in our previous publication (75). As necessary, the lipid fractions will be separated and quantitated by thin layer chromatography or by gas liquid chromatography.

Hexose monophosphate shunt activity will be calculated from glucose utilization and glucose-1- ^{14}C and glucose-6- ^{14}C oxidation by the method of Katz and Wood (76). Glycerinated phagocytes are prepared for enzyme assays by adding 0.6 ml of cell suspension to 1.4 ml of 95% glycerin. Glucose-6-phosphate dehydrogenase will be assayed by measuring NADP^+ reduction at 340 nm in a medium containing 200 μmoles of Tris (pH 7.5), 10 μmoles of MgCl_2 , 0.625 μmole of NADP^+ and 5 μmoles of glucose-6-phosphate in 3 ml. 6-phosphogluconate dehydrogenase will be assayed in a similar reaction mixture where glucose-6-phosphate is replaced with 10 μmoles of 6-phosphogluconate. NADPH oxidase will be assayed by measuring the oxidation of NADPH at 340 nm in a reaction mixture containing 300 μmoles of phosphate buffer at pH 5.5, 1.0 μmole of MnCl_2 , 200 μmoles of ethanol and 0.5 μmole of NADPH in 3 ml (77). D-amino acid oxidase will be estimated by measuring O_2 consumption from D-alanine in a reaction mixture containing 100 μmoles and pyrophosphate buffer, pH 8.3, 0.5 μg catalase,

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10 mμmoles FAD and 60 μmoles D-alanine (78). L-amino acid oxidase will be estimated by measuring O_2 consumption in 0.2 M tris buffer at pH 7.2 containing 0.1 M KCl with 0.1 M l-leucine as substrate (79).

Production of H_2O_2 by intact phagocytes will be determined by an indirect procedure by the oxidation of ^{14}C -formate (80). Direct fluorometric assay for H_2O_2 will also be carried out in cell dialysates by a procedure developed in our laboratory (81). Peroxidase activity will be measured by three different procedures. First, will be the conventional guaiacol oxidase activity by the method of Chance and Mahely which is optimum at pH 7.0 (82). The second procedure will be the decarboxylation of alanine- l - ^{14}C catalyzed by peroxidase at pH 5.5 in presence of Cl^- (83,84). Finally, peroxidase will also be assayed by the catalytic iodination of bacteria by the method of Klebanoff (85). Catalase will be assayed by the perborate method of Feinstein (86).

Lysosomal hydrolytic enzymes will be assayed by conventional techniques. Acid and alkaline phosphatases will be assayed with p-nitrophenyl phosphate as substrate with citrate buffer (pH 4.8) and glycyl-glycine buffer (pH 10.4) respectively. Cathespin will be estimated at pH 3.8 by the method of Adams and Smith (87). Nucleotidase will be assayed by the method of Swenseid et al (88) with adenosine-5 phosphate as substrate. Ribonuclease and deoxyribonuclease will be estimated by the procedure of Folette et al (90). Lysozyme will be assayed by the method of Shngar (91).

(5). Phagocytic and bactericidal studies: Phagocytosis will be measured by incubating viable or heat killed bacteria with the phagocytes (50:1) at 37°C for 30 minutes. Smears from the incubation mixture will be stained by May-Greenwald-Giemsa method. Both the number of phagocytes containing bacteria and the number of bacteria per active phagocyte will be counted to estimate the percent phagocytosis and phagocytic efficiency.

Bactericidal activity studies will be carried out using organisms which include pathogenic clinical isolates, those with pathogenicity enhanced by animal passage and laboratory strains from St. Margaret's Hospital stock culture collection. Logarithmic phase cultures of bacteria will be used. The ratio of particles to phagocytes will vary with the experiment. A typical experiment will contain at least two tubes, one being the bacterial control, the other containing both the phagocytes and bacteria. After suitable incubation periods at 37°C, aliquots will be removed from each tube. Those tubes to be evaluated for bactericidal activity will be diluted initially in 5% saponin to lyse the phagocytes. Subsequent dilutions will be in 0.9% NaCl. Aliquots of these dilutions will be plated out in trypticase soy agar using a semi-micro pour plate procedure. These plates will be incubated at 37°C and colonies will be counted by conventional methods.

Peroxidase containing subcellular fractions will also be assayed for bactericidal activity as described for the intact cells. These experiments will be carried out in the presence of a H_2O_2 producing system (glucose-glucose oxidase) and either Cl^- or I^- as halide, at pH 5.5.

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10. Space and facilities available:

The complete facilities of St. Margaret's Hospital will be available for this study. These facilities include:

1. A suite for tissue culture equipped with a walk-in incubator and an explant room under positive pressure. Supporting equipment for this laboratory includes: A Reichert time lapse cinema-micro-photography apparatus, dissection microscope, microscope with phase contrast and inverted microscope, drying ovens, autoclave, refrigerator and deep freeze.

2. A virology laboratory includes a Spinco Model L ultracentrifuge LKB immunoelectrophoresis and immunodiffusion analysis equipment, hood with ultra-violet lamp, pH meter, micromanipulator, refrigerators and freezers.

3. Biochemical facilities include two laboratories equipped with a Spinco Model E analytical ultracentrifuge, Nikon microcomparator, refrigerated centrifuges, International PR2 and Servall KSB-R with attachment for continuous flow centrifugation, pH stat, Gibson model D electrophorator, facilities for paper chromatography, paper electrophoresis and curtain electrophoresis, electric deslatter, Beckman DU and DB spectrophotometers with fluorescence attachment, and amino acid analyzer, fraction collectors, two Warburg constant volume respirometers including a refrigerated model, GC-5 steroid analyzer with a Linear Recorder and a disc integrator, radio-chemical facilities including a Tricarb liquid scintillation spectrometer of the 3000 series and other supporting equipment including pH meters, water baths, analytical balances, Buchler continuous flow evaporators, water deionizers, freeze-dry apparatus, all-glass water still, Buchler constant current power supply of 1000 volt capacity and cold room with walk-in freezer.

4. A laboratory housing the RCA-EMU-3 electron microscope is available for this study and is equipped with dark room for film development and a separate laboratory for the preparation of specimens. A Porter-Blum microtome is available for ultrathin sections along with other supporting equipment.

5. Tissue processing laboratory equipment with routine and experimental histology and histochemistry. The equipment in this laboratory consists of an Autotechnicon, paraffin ovens, microscopes, a cryostat, microtome for frozen section, rotary microtomes, frozen section microscope and deep freeze.

6. Clinical laboratories including blood bank, hematology, bacteriology, biochemistry, urinalysis, endocrinology and immunology sections will be completely available to us for this project.

7. Animal rooms completely air conditioned and equipped to handle infectious and non-infectious experiments, an associated experimental surgery room and a room housing an Andrex Model 3001, 280 kVp x-ray apparatus.

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8. An autopsy room with all facilities necessary for teaching and practice.

9. A conference room furnished with projection apparatus for tissue and graphic slides, screen, etc. is available.

10. A larger room equipped with projection apparatus is available for meetings and seminars.

In addition, the facilities required to obtain and prepare patient specimens will be available at the Veterans Administration and Boston City Hospitals. Also, Dr. Joseph Vitale's research laboratories at Boston City Hospital will be utilized as necessary.

11. Additional facilities required:

None.

12. Biographical sketches of investigator(s) and other professional personnel:

Please see curriculum vitae for A.J. Sbarra, R.J. Selvaraj and J.J. Vitale.

13. Publications:

Please see enclosed reprints.

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* 18.

14. First year budget:

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s)
even if no salary requested)

% time

Amount

A.J. Sbarra, Principal Investigator

15

J. Vitale, Nutritionist

5

R.J. Selvaraj, Biochemist

100

To be recruited, Res. Tech.

100

To be recruited, Res. Tech.

100

Secretary, lab aide

50

Technical

Sub-Total for A

B. Consumable supplies (by major categories)

Chemicals, radiochemicals

3,000

Biologicals

2,000

Glassware

1,000

Sub-Total for B

6,000

C. Other expenses (itemize)

Publication costs, travel to attend scientific meetings,
maintenance of existing equipment, transportation of
samples.

Sub-Total for C

3,000

Running Total of A + B + C

54,000

D. Permanent equipment (itemize)

Oxygraph with recorder

3,000

Sub-Total for D

3,000

E. Indirect costs (15% of A+B+C)

E

8,100

Total request

65,100

15 Estimated future requirements

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2	R	6,600	3,300	-	8,910	68,330
Year 3		7,260	3,630	-	10,301	75,641

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects

CURRENTLY ACTIVE			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The effect of x-irradiation on the biochemical changes of mammalian cells in the absence and presence of particulate material	AEC AT(11-1) 3517	28,000	August 7, 1972- August 6, 1973

PENDING OR PLANNED			
Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Metabolism of neoplastic cells during phagocytosis	NIH CA 05317	59,560	September 1, 1973- August 31, 1974

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

St. Margaret's Hospital

Mailing address for checks
90 Cushing Avenue

Boston, Massachusetts 02125

Principal investigator

Typed Name Anthony J. Sbarra, Ph.D.

Signature [Signature] Date 5/30/73

Telephone 617 436-8600 237
Area Code Number Extension

Responsible officer of institution

Typed Name Sister Mary Bernadette Doyle

Title Administrator, St. Margaret's Hospital

Signature [Signature] Date 5/30/73

Telephone 617 436-8600 202
Area Code Number Extension

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CURRICULUM VITAE

NAME: Anthony J. Sbarra

ADDRESS: REDACTED

BIRTHPLACE: REDACTED

BIRTHDATE: REDACTED

MARITAL STATUS: REDACTED

EDUCATION:

<u>School</u>	<u>Degree</u>	<u>Date</u>	<u>Major</u>
Siena College	B.S.	REDACTED	Biology and Chemistry
University of Kentucky	M.S.	REDACTED	Bacteriology
University of Tennessee	Ph.D.	REDACTED	Bacteriology

SOCIETIES:

REDACTED

RESEARCH INTERESTS:

Biological and biochemical aspects of host-parasite interactions.

POSITIONS HELD:

Associate Biologist, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1953-1956.

Lecturer in Bacteriology, Department of Bacteriology, University of Tennessee, 1954-1955.

Research Fellow, Research Associate, and Instructor in Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts, 1956-1961.

Associate Director, Department of Pathology and Medical Research, St. Margaret's Hospital, Boston, Massachusetts, 1959-

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Assistant Professor of Bacteriology, Tufts University School of Medicine,
Boston, Massachusetts, 1959-1963.

Assistant Professor, Associate Professor and Professor of Obstetrics and
Gynecology (Microbiology), Tufts University School of Medicine, Boston,
1963-

Editor, Biochemistry Section, Journal of the Reticuloendothelial Society,
1966-

Secretary, Reticuloendothelial Society, 1970.

President, Boston Bacteriologists Club, 1970.

Vice-president, Reticuloendothelial Society, 1971.

President, Reticuloendothelial Society, 1972.

Editorial Board, Journal of Infection and Immunity, 1973.

Councilor, Reticuloendothelial Society, 1973.

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PUBLICATIONS

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CURRICULUM VITAE

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EDUCATION:

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Madras University

B.Sc.

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Chemistry

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Biochemistry

SOCITIES: REDACTED

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RESEARCH INTEREST:

Biochemistry of host-parasite relationships

POSITIONS HELD:

Research Assistant, ICMR, Nagpur, India, 1957-1960.

Research Assistant, McGill University, Canada, 1960-62.

Research Associate, Tufts University School of Medicine, Boston, Mass., 1963-65.

Senior Biochemist, Department of Pathology and Medical Research, St. Margaret's Hospital, Boston, Mass., 1965-67.

Assistant Professor of Obstetrics and Gynecology (Biochemistry), Tufts University School of Medicine, Boston, Mass., 1965-67.

Scientific Pool Officer, CSIR, Lucknow, India, 1967-68.

Research Officer, NIN, Hyderabad, India, 1968-69.

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Senior Research Officer, NIN, Hyderabad, India, 1969-72.

Senior Biochemist, Department of Pathology and Medical Research, St. Margaret's Hospital, Boston, Mass., 1972-

Assistant Professor of Obstetrics and Gynecology (Biochemistry), Tufts University School of Medicine, Boston, Mass., 1972-

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PUBLICATIONS

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2. Selvaraj, R.J. Studies on the intermediary metabolism of glycine. Ph.D. Thesis, McGill University, Montreal, Canada, 1962.
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20. Selvaraj, R.J. Phagocytic responses in protein-calorie malnutrition. In: Symposium on Metabolic Responses to Protein-Calorie Malnutrition. Proc. First Asian Cong. of Nutr., Hyderabad, India, 1971.
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CURRICULUM VITAE

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EDUCATION:

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Northeastern University

B.S.

New York Univ. Medical School

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Harvard School of Public Health

Sc.D.

Universidad de Antioquid (Colombia)

M.D.

Biology

Physiology

Nutrition, Biochemistry

SOCITIES:

RESEARCH INTEREST:

Nutrition as dealing with malnutrition and alcoholism and folate and vitamine deficiencies.

POSITIONS HELD:

Toxicologist, Massachusetts Department of Public Safety, 1949.

Research Assistant, Mallory Institute of Pathology, Boston, City Hospita, 1951-60.

Research Associate in Nutrition, Harvard School of Public Health, 1951-61.

Assistant Professor of Nutrition, Visting Professor of Nutrition, School of Medicine, Universidad del Valle, Cali, Colombia, 1960-62.

Scientific Director, Cali-Harvard Nutrition Project, Universidad del Valle, Cali, Colombia, 1960-62.

Co-director, Tufts Antioquia Collaborative Nutrition Program, 1963-

Co-scientific Director, Medellin, Colombia-Harvard Nutrition Cooperative Program, 1964-66.

Professor of Nutrition, College of Agriculture, University of Wisconsin, 1966.

Research Associate, School of Medicine, University of Wisconsin, 1967.

Senior Pathologist, Research, Mallory Institute of Pathology, Boston City Hospital, 1967.

Professor of Nutrition, Department of Preventive Medicine and Pathology and Director of Nutrition Programs, Tufts University School of Medicine, Boston, Mass., 1967-1972.

Consultant in Nutrition, St. Margaret's Hospital, Boston, Mass., 1969-

Professor of Pathology and Community Medicine, Boston University School of Medicine, Boston, Mass., 1972-

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Vitale, J.J., Hegsted, D.M., DiGiorgio, J. and Zamcheck, N. Inter-relations between pantothenic acid, protein and calorie intakes with respect to respiration and morphology of duodenal mucosa. *Metabolism*, 2, 367, 1953.

Vitale, J.J., Zamcheck, N., DiGiorgio, J. and Hegsted, D.J. Effects of amino-protein administration on the respiration and morphology of the gastrointestinal mucosa of rats. *J. Lab. Clin. Med.*, 43, 583, 1954.

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Ghitis, J. and Vitale, J.J. Cali-Harvard Nutrition Project. V. Anemias of protein malnutrition. Postgrad. Med., 34, 300, 1963.

Vitale, J.J., Restrepo, Va., Velez, H., Riker, J.B. and Hellerstein, E.E. Secondary folate deficiency induced in the rat by dietary iron deficiency. J. Nutr., 88, 315, 1966.

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#936 - VMDIC

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THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

August 6, 1973

Grant Application No. 936
PULMONARY

To: The committee comprising Drs. Bing, Loosli,
Sommers, and Wyatt

Subject: Branislav Vidic, S.D., Georgetown University School
of Medicine, D.C.
New application No. 936
"The Effect of Cigarette Smoke on Lung Metabolism"

History

This proposal was Case No. 206. Dr. Lisanti was requested to visit, with authorization to suggest formal application if appropriate. He did so on July 25, 1973.

Application No. 936 requests \$40,738 plus two additional years.

Document Submitted

Attached is application dated July 31, 1973, with three appendices.

Comment

CTR Grantee Hamosh ("The Effect of Smoking on Small Airways") is committed to 15% of his time in connection with the enclosed proposal. Dr. Hamosh's wife, Dr. Margit Hamosh (an NIH biochemist), is listed as a Consultant.

FWM

F.W.N.

FWN:wg
Encl.

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Comm.

Dr. Bing
Dr. Loosli
Dr. Sommers
() Wyatt

PULMONARY

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
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(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

71936
Date: July 31, 1973

1. Principal Investigator (give title and degrees):
Branislav Vidic, S.D., Assoc. Prof. Anatomy; Director of Project
Paul Hamosh, M.D., Ass't. Prof. Physiology & Biophysics and Medicine;
Co-Principal Investigator
Henry Yeager, M.D., Ass't. Prof. Medicine; Consultant
Margit Hamosh, Ph.D., Consultant
2. Institution & address:

Georgetown University School of Medicine
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Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:

4. Short title of study:

The Effect of Cigarette Smoke on Lung Metabolism

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

The aim of this research is to study the effect of cigarette smoke, filtered and unfiltered, on the biosynthesis and function of surfactant and connective tissue (elastin and collagen). An integrated approach, using the tools of physiology, morphology and biochemistry is presented. Rats will be chronically exposed to cigarette smoke and then subjected to three main experimental designs. The first aims to study the incorporation and turnover of labeled substrates presented to the intact animal. The second design calls for isolation of the lung into a ventilated, perfused preparation. This preparation can be "stressed" both mechanically (forced ventilation) and biochemically (substrate deprivation). We shall study the effect of these acute "stresses" on the biosynthesis and turnover of substrates in lungs of chronically smoked and control rats. The third design involves isolation of the great alveolar cell and the study of in vitro surfactant synthesis.

The major objective of this research is to establish the site, sequence and extent of tobacco smoke effect of the biosynthetic pathways responsible for the maintenance of integrity of structure and proper function (elastic recoil) of the lungs.

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8. Brief statement of working hypothesis:

2.

Rats exposed to chronic cigarette smoke might show detectable changes in 1) biosynthesis of surfactant; 2) biosynthesis of elastin and collagen; 3) chemical and functional response patterns to acute mechanical or chemical stress.

9. Details of experimental design and procedures (append extra pages as necessary)

See appended.

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9. Background, details of experimental design and procedure

GENERAL BACKGROUND

The effect of cigarette smoke on human and animal lung has been widely investigated. It has been shown that chronic exposure to cigarette smoke will result in changes in the airways: chronic bronchitis (1). The link between smoking and emphysema is less well documented. The frequent association between chronic bronchitis and emphysema in smokers (2) suggests some causal relationship. The present tendency is to shift the initial changes occurring in the pathogenesis of chronic obstructive lung disease further toward the periphery of the lung, mainly the small airways (3). Little work has been done on the effect of cigarette smoke on the function of the alveolar epithelium or the biosynthesis of surfactant, elastin and collagen. Quoting from the Task Force Report on Respiratory Diseases of the National Institutes of Health (4): "Information is badly needed concerning the mechanism of cigarette smoking damage. Here, it would be well to take advantage of the many advances in cellular biology that have been made in the past ten years. Some such studies are already in progress, including investigations concerning clearing mechanisms, ciliary action, and quantitative studies of bronchial cellular change. What have not been looked at carefully have been the determinants of individual response, including metabolism of cigarette smoke products, enzyme effects, variations in tissue repair."

In their conclusion it is also stated: "Research should be addressed to fundamental studies of the mechanism of damage from cigarette smoking..."

SPECIFIC BACKGROUND

The great alveolar cell (type II) in the alveoli is a secretory cell, whose main function is the biosynthesis and secretion of surfactant, a substance essential for the proper function of the lung as a mechanical pump. The elastic behavior of the lung is dependent on two factors: 1) adequate or functionally effective surfactant and 2) integrity of the connective tissue components in the alveolar wall, such as collagen and elastin. Very little is known on the effect of smoke on surfactant function and synthesis and practically nothing of its effect on the biosynthesis of connective tissue.

1. Effect of cigarette smoke on surfactant function and synthesis

- a. Cigarette smoke alters the surface characteristics of lung extract (5,6,7,8)
- b. The biosynthesis of lecithin was studied in cigarette smoking dogs (9). This study is unacceptable as a good

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physiologic study because of the large dose and short duration of cigarette exposure. Nevertheless, it showed a significant decrease in choline and phosphate incorporation into lung lecithin.

- c. The removal of used-up surfactant by alveolar macrophages and the effect of smoking on this process has attracted wide attention lately. However, this problem is outside the scope of our intended investigation.

2. The effect of cigarette smoke on connective tissue metabolism of the lung. This area of investigation is unexplored. Only the level of hydroxyproline, a marker of collagen metabolism (10) and a nonspecific "biochemical screen" (11) were measured after whole cigarette smoke exposure in mice. Unfortunately, the information about the normal biosynthesis of elastin and collagen in lung tissue is at best sketchy (12).

SIGNIFICANCE

Maximum expiratory flow, the major mechanical determinant of lung function is directly proportional to lung recoil pressure and inversely proportional to airway resistance. Lung recoil pressure is determined by surface tension which contributes about two thirds, and tissue elastic recoil which contributes about one third to it. Lung recoil pressure is also a function of lung inflation and the elastic properties of the lung are expressed in terms of volume/pressure relationship (lung compliance). Surface tension is modified by the presence of a surface active layer in the alveoli, called surfactant. This substance is responsible for the variation of surface tension with volume. Surface tension increases with lung volume and drops significantly at low lung volume. This mechanism is essential to prevent the collapse of lung regions (atelectasis) at the end of each expiration and therefore constitutes an essential mechanism for survival (13).

In emphysema, lung recoil is severely decreased by impaired tissue elasticity and probably by altered surfactant. The basic question is: What are the factors operative in causing these changes? In addition to this basic question we pose an additional one: Is cigarette smoke a primary, a contributory or an innocent factor in the pathogenesis of emphysema? These fundamental questions have not been answered by previous research. If cigarette smoke does have an effect, the mechanism is still unclear. This research is a step by step integrated approach to answer these questions.

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References

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9. J.A. Balint, S. Bondurant and E.C. Kyriakides. Lecithin biosynthesis in cigarette smoking dogs. *Arch. Intern. Med.* 127:740, 1971.
10. H. Rosenkrantz, H.J. Esber and R. Sprague. Lung hydroxyproline level in mice exposed to cigarette smoke. *Life Sciences* 8:571, 1969.
11. H. Rosenkrantz and R. Sprague. Biochemical screen to investigate whole smoke and vapor phase effect in mice. *Arch. Environ. Health* 18:917, 1969.
12. R.G. Crystal, K.H. Bradley, and S.D. McConnell. Changes in lung collagen synthesis with age. Annual Meeting of American Thoracic Society, May 21-23, 1971 (abstract).
13. J. Mead. Mechanical properties of lungs. *Physiol. Rev.* 41:281, 1961.

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TIMETABLE

General. All of the equipment except for the exposure apparatus and most methods in design one and two are already operational.

First Year. Recruitment of personnel, establishment of experimental routines and assembly of exposure apparatus will take between three and six months. However, work will proceed on acquiring basic information about lung metabolism (such as present in appendix I). The second half of the year will be used for studies of design one and preliminary studies of design two.

Second Year. Most of the work will be on design two (i.e. using the isolated/perfused lung preparation). The effect of forced ventilation, substrate deprivation, perfusion pressure etc. in smoked and control lungs will be investigated. Design three will be developed.

Third Year. Mostly work on isolated type II cells on the bio-synthesis, location, storage and secretion of surfactant.

EXPERIMENTAL DESIGN

1. Selection of experimental animals. The material for this study will consist of caesarian-delivered adult male Sprague-Dawley rats. This species has been selected because of convenience for purchasing and maintenance. Caesarian delivered rats have a far lower incidence of lung infection than normally delivered. The animals will be individually maintained for at least two weeks in an isolated chamber under optimal conditions (with controlled humidity and temperature) and on laboratory Purina Chow diet ad libitum. Healthy animals without signs of respiratory disease will be divided into three groups: experimental, sham control and cage control. The experimental group will be further subdivided into a number of subgroups. Each subgroup consisting of six animals will be exposed to cigarette smoke for differently designed periods of time. Two other subgroups of six animals, sham and cage controls respectively, will be considered in relation to every experimental subgroup.

2. Exposure to cigarette smoke. In order to make anticipated results of this proposal better comparable to other similar studies, we are proposing that the "smoking machine" be supplied from the Council for Tobacco Research - U.S.A. In case that such an arrangement cannot be made, we will construct our own device that would best fulfill Requirements For Any Mechanical Arrangement For Exposure Of Animals To The Inhalation Of Cigarette Smoke Under Conditions Comparable To Those of Human Smoke Exposure. The "reference" cigarette from the University of Kentucky will be used

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for the entire project. Some experimental subgroups will be exposed once daily (9 a.m.) for one hour, others twice daily (9 a.m. and 5 p.m.) for one hour to whole cigarette smoke. Still other subgroups will be exposed to cigarette smoke filtered through a Cambridge filter. Sham control subgroups will be enclosed in the same chamber through which the room air instead of cigarette smoke will be circulated for the same lengths of time as for the corresponding experimental subgroups. Since the mode of exposure has not been finalized the duration of exposure will be determined after resolution of this question.

3. Experimental design, schematic presentation. We propose three approaches for this study. The first approach (design one) is designed to study the dynamics and morphology in the intact animal. The second approach (design two) utilizes our ability to keep the lungs viable and functioning in an isolated system. This system provides controlled ventilation and perfusion. It also provides the opportunity to "stress" these lungs by forced ventilation, by substrate deprivation etc. This enables us to compare the performance of lungs from smoked and control rats under adverse conditions and thus test "in vitro" multiple etiology. The third approach (design three) involves the separation and isolation of pure type II cells. Whereas the previous two techniques are available at least partially to us, the third approach has not been developed yet and needs to be introduced. It certainly promises to be the method of choice in analyzing processes at the cellular level.

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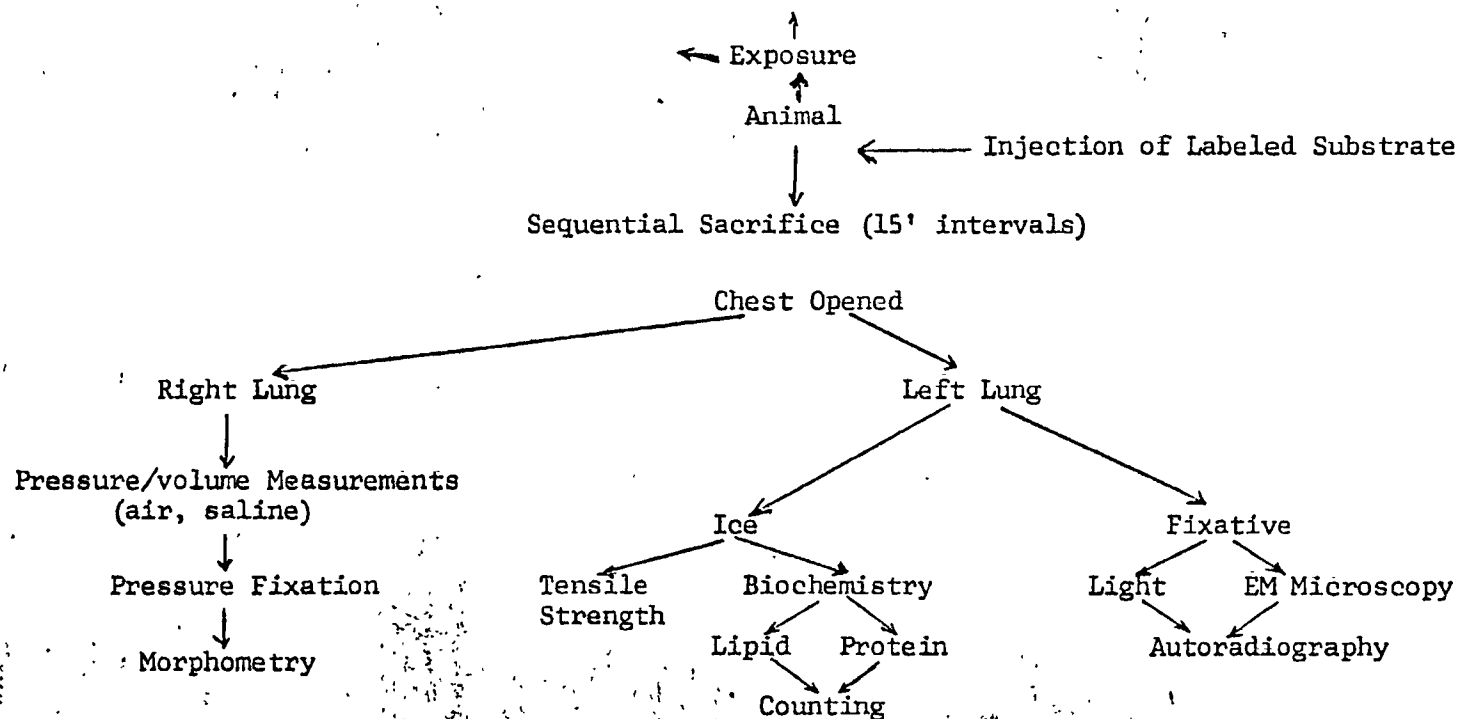
EXPERIMENTAL DESIGN ONE

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Unfiltered Smoke

Filtered Smoke

Control



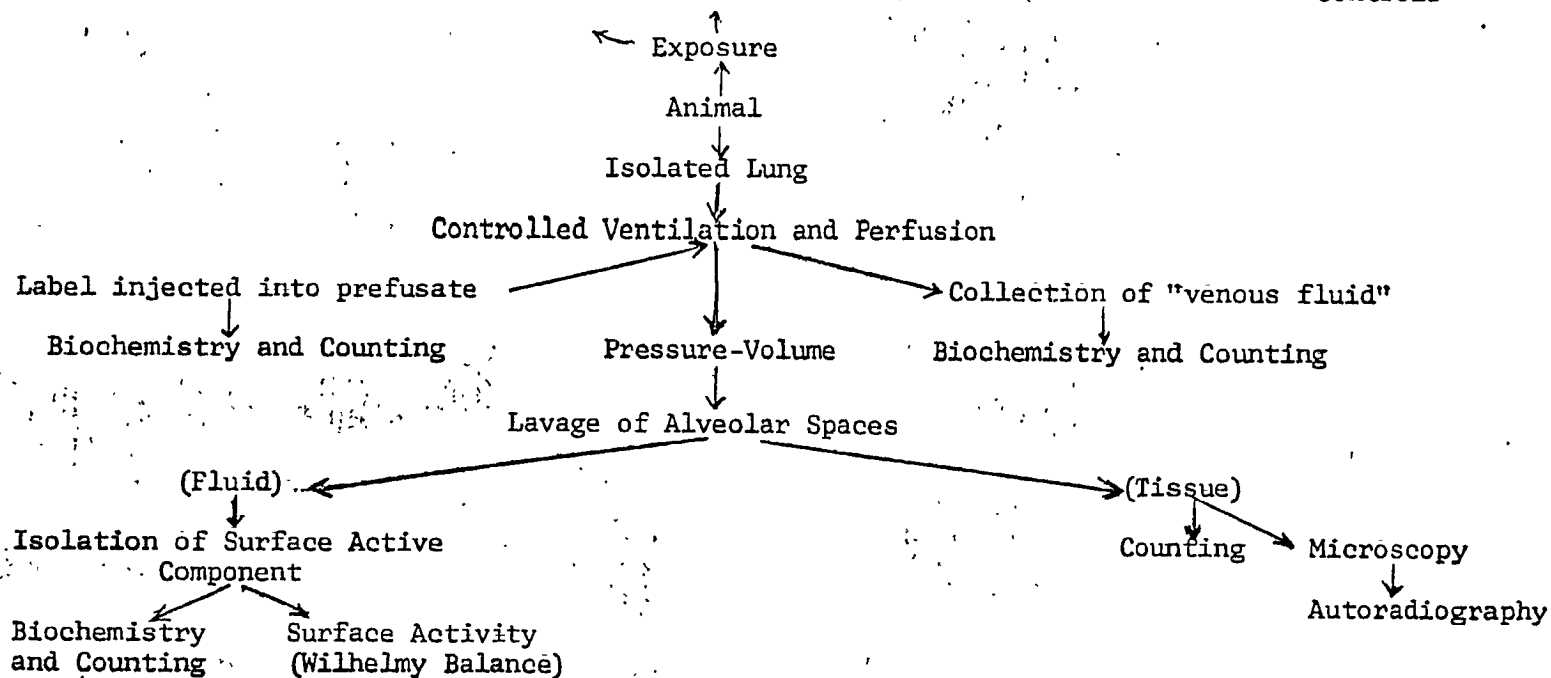
EXPERIMENTAL DESIGN TWO

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Unfiltered Smoke

Filtered Smoke

Controls



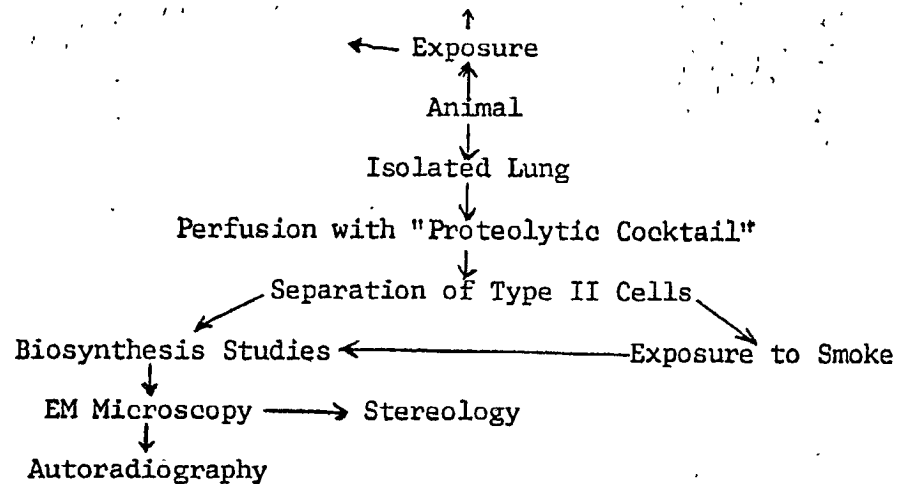
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EXPERIMENTAL DESIGN THREE

Unfiltered Smoke

Filtered Smoke

Controls



It is not implied that all steps will be incorporated in every sequence of experiments. Each design could be performed in several stages. This will depend on logistics and skill of the investigators and their assistants. However, this team of investigators have already performed work of this nature (Hamosh and Hamosh. Lung lipoprotein lipase, effect of starvation. See appended manuscript.)

NARRATIVE DESCRIPTION OF EXPERIMENTAL DESIGNS

A. Design One

Animals (controls and exposed) will be injected into the tail vein with radioactive labeled substrates, mostly C^{14} and H^3 palmitate and leucine (see procedure). The chest will be opened and the specimen obtained from the left lung and distributed in appropriate fixatives for EM or frozen (cooled) and processed for extraction, homogenization or sliced. The right main bronchus will be then cannulated and the lung inflated to the desired transpulmonic pressure and the deflation pressure volume relationship measured. Following this the lung will be pressure fixed with formaldehyde, embedded and sliced. The microscopic slides will be subjected to morphometric and autoradiographic evaluation.

B. Design Two

The animal will be anesthetized by intraperitoneal injection of Pentobarbital and the trachea cannulated. The pulmonary artery will be cannulated through the right ventricle and the left atrium cannulated for collection. The specimen will be transferred into the box designed for controlled perfusion and ventilation at $37^{\circ}C$. It is also equipped for continuous monitoring of transpulmonic pressure, so that the pressure/volume relationship can be monitored. After establishing a baseline under controlled conditions, the labeled substrate is added to the perfusate. The "venous" part of this non-circulating perfusion will be collected in test tubes at equal time intervals and prepared for the scintillation counter. Comparing the activity of the perfusate to the activity of the sequential collection, the dynamics of the turnover of the labeled substance can be determined by calculation. Surfactant will be both present in the tissue and secreted to the alveolar surface. Therefore, at the termination of perfusion the lung will be "washed out" by saline to harvest the surface active fraction and determine the radioactivity. Specimens of the tissue will be taken for autoradiography and extraction of lipid, to determine the activity and its location in the tissue.

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C. Design Three

This design calls for the separation and harvest of individual type II cells and their isolation in relatively pure and viable short term cultures. The feasibility of this has been demonstrated by Gould et al.* To manipulate pure cell population is ideal for determining the dynamics of intracellular processes. It is possible to study the comparative behavior of cells from smoked vs. non-smoked animals. It would also be of great interest to expose these cells in vitro to cigarette smoke.

PROCEDURES

A. Electron Microscopy

Ultrastructural Methods. Tissue samples from lungs in vivo (design one), perfused lungs (design two) and in vitro cultured cells (design three) will be fixed in an aldehyde mixture (1) for two hours at 4°C and washed overnight in an appropriate buffer. Following the post-fixation in one percent osmium tetroxide, the samples will be dehydrated, embedded in Epon and sectioned with a diamond knife in 300-600 Å thin sections. Some grids (designs one, two and three) will be treated conventionally with heavy metals, whereas the other grids (designs one and two) will be processed according to phosphotungstic acid (10% aqueous solution, pH 1.5) procedure of Kay (2) and silver tetraphenylporphinesulfonate technique of Albert and Fleischer (3) for demonstration for collagen and elastin fibers respectively.

Autoradiographic Methods (4). Tissue samples from lungs in vivo (design one), perfused lungs (design two) and in vitro cultured cells (design three) will be fixed in a 2% osmium tetroxide buffer with veronal acetate to pH 7.2 for two hours at 4°C and washed overnight in an appropriate buffer. Following the block staining in 1% uranyl acetate, dehydration and embedding in Epon, tissue samples will be sectioned with a diamond knife in 600-900 Å thin sections and mounted on grids previously coated with collodion film baked by a thin carbon layer. The grids will be exposed to the photosensitive emulsion (Ilford L-4) diluted one to four with distilled water for approximately six months, developed in Microdal X for 5 minutes, washed, fixed in Kodak rapid fixer for 5 minutes and washed again in running and distilled water for 5 and 2 minutes respectively.

Electron microscopy and photography will be carried out with the aid of an AEI-EM 8 electron microscope.

* Science 178:1209, 1972

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References

1. S. Ito and M.J. Karnovsky. J. Cell Biol. 39:168A, 1968.
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3. E.N. Albert and E. Fleischer. J. Histochem. Cytochem. 18: 697, 1970.
4. L.G. Caro, R.P. van Tubergen and J.A. Kolb. J. Cell Biol. 15:173, 1962.

B. Biochemistry of Connective Tissue

Analytical studies. Segments of lung will be pooled, weighed and assayed for DNA by the method of Schmidt (1), for hexosamine by a micromodification of the method of Randle and Morgan (2) and for hydroxyproline by the method of Woessner (3). All determinations will be expressed as micrograms per milligram of dry weight and will be repeated at least two times on each sample for verification.

Assays for incorporation of radioisotopes. The samples (200 mg wet weight) will be sliced 1 mm thick with a McIlwain tissue chopper and incubated at 37°C for two hours in tubes containing 50 microcuries of thymidine-³H and 20 microcuries of glycine-¹⁴C; or 50 microcuries of cytidine-³H and 20 microcuries of ³⁵SO₄. After incubation the samples will be assayed for radioactivity (4), adjusted for simultaneous counting of double-labeled samples (5). The values obtained will be recorded as counts per minute (CPM) per milligram of dry weight and as CPM per milligram of DNA.

It will then be possible to calculate data for lung content of DNA, hexosamine, hydroxyproline, and hexosamine/hydroxyproline ratios. From the radioisotope incorporation data it will be possible to get estimates of the rates of DNA, RNA, Protein, and polysaccharide synthesis.

References

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2. C.J.M. Randle, and W.T.J. Morgan. Biochem. J. 61:586, 1955.
3. Woessner, J.F., Jr. Arch. Biochem. Biophys. 93:440, 1961.
4. H. Yeager, Jr. and P.S. Hicks. Proc. Soc. Exp. Biol. Med. 141:1, 1972.
5. J.B. Birks. The Theory and Practice of Scintillation Counting. New York: Pergamon Press, 1964.

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C. Biochemistry of Lipids

Labeled palmitic acid and labeled leucine will be used as precursors in the biosynthesis of lung lipid (with special emphasis on surfactant) and protein. The studies will be carried out in vivo and in the isolated perfused lung preparation.

Palmitic acid will be prepared for intravenous administration or tissue perfusion by the method of Darrah and Hedley-Whyte (1). Lipids will be extracted according to Folch et al. (2) and Dole (3). Total lipid concentration will be determined according to Rapport and Alonzo (4) and Dole and Meinertz (5). Phospholipid concentration will be determined according to Bartlett (6). The different phospholipid fractions will be separated by column and thin layer chromatography according to Mason et al. (7), Young and Tierney (8), Mangold (9) and Balint et al. (10).

The neutral lipid fractions will be separated by chromatographic procedures according to Hamosh and Scow (11) and Creech (12). Radioactivity in the isolated lipid fractions will be determined according to Hamosh et al. (13) and Snyder (14).

References

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2. J. Folch, M. Lees and G.H. Sloam-Stanley. J. Biol. Chem. 226:497, 1957.
3. V.P. Dole. J. Clin. Invest. 35:150, 1956.
4. M.M. Rapport and M. Alonzo. J. Biol. Chem. 217:193, 1959.
5. V.P. Dole and H. Meinertz. J. Biol. Chem. 235:2959, 1960.
6. G.R. Bartlett. J. Biol. Chem. 234:466, 1959.
7. R.J. Mason, G. Huber and M. Vaughan. J. Clin. Invest. 51:68, 1972.
8. Young, S.L. and D.F. Tierney. Am. J. Physiol. 222:1539, 1972.
9. Mangold, H.K. In Thin-Layer Chromatography. A Laboratory Handbook, ed. E. Stahl. New York: Academic Press, 1965, pp 137-86.
10. J.A. Balint, S. Bondurant and E.C. Kyriakides. Arch. Intern. Med. 127:740, 1971.
11. Hamosh, M. and R.O. Scow. J. Clin. Invest. 52:88, 1973.
12. B.G. Creech. J. Am. Oil Chem. Soc. 38:540, 1961.
13. M. Hamosh, T.R. Clary, S.S. Chernick and R.O. Scow. Biochim. Biophys. Acta 210:473, 1970.
14. F. Snyder. Anal. Biochem. 9:183, 1964.

D. Physiology

Determination of the elastic properties of intact lung

1. Pressure-volume measurements. The lung will be inflated to a transpulmonic pressure of 25 cm H₂O and the lung volume at

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this distending pressure called "vital capacity". The lung will be deflated with a syringe by decrement of about one tenth of the vital capacity and the pressure at each volume recorded. This procedure will be repeated three times and the resulting curves plotted as volume/pressure curves. Pressure will be measured by a Hewlett-Packard 267B transducer, the signal amplified by a Hewlett-Packard 8805A carrier amplifier, the signal recorded on a Hewlett-Packard recorder. In saline filled lungs the pressure will be measured by a Statham P23V transducer.

2. Isolated lung preparation. After cannulation of the trachea, pulmonary artery and left atrium, the lung is suspended in a chamber thermostated at 37°C and saturated with water vapor. The perfusate has the basic composition of 4% albumin in Krebs-Ringer solution contained in a 37°C bath. The radioactive substrates are delivered into the line leading to the pulmonary artery with a metering pump (Sage Co.) The fluid from the left atrium is collected by gravity into iced test tubes in a modified fraction collector. The tracheal cannula is connected to a rodent respirator (Harvard Instrument Co.) with controlled volume and frequency. The chamber serves also as a volume plethysmograph and lung volume and transpulmonic pressure can be continuously monitored. Perfusate flow is monitored by a drop-counter on the collection site. Perfusion pressure is determined by the height of the perfusion unit, but pulsatile flow can be also generated by a peristaltic pump. The lung is suspended from the force-displacement transducer, so that lung weight can be also monitored continuously.

3. Determination of tensile strength. A longitudinal strip of lung tissue, trimmed and oriented, will be constrained on both ends and connected to a force-tension transducer and a linear displacement transducer. Stress and Strain will be measured and yield stress determined (Martin et al.)* This measurement is a good index of tissue elasticity.

4. Determination of area dependent surface activity. The surface active fraction from the lavage fluid will be separated by the method of Dickie et al. **. The fraction will be spread over saline and the activity determined as a function of area using a modified Wilhelmy balance.

*T. Sugihara, C.J. Martin and J. Hildebrandt. J. Appl. Physiol. 30:874, 1971

** K.J. Dickie, G.D. Massaro, V. Marshall and D. Massaro. J. Appl. Physiol. 34:606, 1973.

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E. Morphometry and Stereology

For rapid evaluation of structural changes a computer technique known as pattern recognition based on the principle of evaluation of rapid electronic scanning (see Appendix II) will be used. In addition we are now in the process of developing a method of stereology for quick determination of the volume of inclusion bodies, such as lamellar bodies. The lamellar body is the storage unit for surfactant and determination of their size and quantity can be a useful index of intermediary metabolism of surfactant (Massaro*).

* G.D. Massaro and D. Massaro. J. Clin. Invest. 52:566, 1973.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

See appended.

11. Additional facilities required:

12. Biographical sketches of investigator(s) and other professional personnel (append):
appended.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).
Appended.

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10. FACILITIES AVAILABLE

A. In the Department of Anatomy (Dr. Vidic)

1. A multidisciplinary laboratory fully equipped for microdissection and light microscopy
2. A fully equipped laboratory for processing of tissues and a fully equipped laboratory for cutting of tissues for electron microscopy and autoradiography
3. A fully equipped laboratory for initiation and maintenance of tissues in vitro
4. A fully equipped darkroom for electron and light microphotography
5. Diamond knife
6. LKB - automatic ultra-microtome
7. AEI-EM 8 electron microscope

B. In the Department of Physiology and Biophysics (Dr. Hamosh)

1. A biochemistry laboratory, about 200 sq. ft. with specialized bench furniture, refrigerator, centrifuge, pH meter and perfusion apparatus.
2. A laboratory for lung mechanics of 200 sq. ft. with all the necessary transducers, amplifiers, recorders etc.
3. A laboratory for animal work, approx. 400 sq. ft with capabilities of housing, exposing, operating, etc.
4. Office space, about 200 sq. ft. to house all data processing equipment
5. Access to and regular use of liquid scintillation counters, preparatory centrifuges, spectrophotometers, cold room, etc. all in the department of physiology
6. In the National Biochemical Research Foundation, affiliated with the department and housed in the same building, access to IBM 360/44 computer with all accessories, primarily intended for pattern recognition work but also available for statistical use.

C. In the Department of Medicine (Dr. Yeager)

The laboratory area has about 320 sq. ft of laboratory space with appropriate sinks and utilities and 200 sq. ft of office space. There is access to liquid scintillation counters in the Department of Nuclear Medicine and Pharmacology and to a preparative ultra-centrifuge in the Pharmacology Department.

Equipment in the laboratory includes:

1. Spectrophotometer
2. pH meter
3. Vortex Genie Mixer
4. Homogenizer
5. Analytical Balance

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6. Refrigerated centrifuge etc.

D. General Facilities

1. The "Vivarium". Accredited animal facility with resident veterinarian and animal care personnel with easy capability to house and care for animals projected in this study.
2. A central facility for storing and dispensing radioactive materials.
3. In addition to the above the university is equipped with a modern library, medical illustration service and data processing unit to handle all bibliographic and publication requirements.

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BIOGRAPHICAL SKETCH

Mailing Address: Branislav Vidic, S.D., Department of Anatomy
Georgetown University School of Medicine,
3900 Reservoir Rd., NW, Washington, D.C. 20007

Date and Place of Birth:

Citizenship:

REDACTED

Marital Status:

Languages Spoken: English, French, German, Russian, Serbo-Croatian

Undergraduate Education: Sremska Mitrovica, Yugoslavia

Graduate Education: Faculty of Stomatology, University of Belgrade,
Yugoslavia. Doctor of Stomatology 1959

Positions Held: Assistant in Anatomy, School of Medicine, University
of Novi Sad, Yugoslavia, 1960-1962.

Visiting Assistant in Anatomy, School of Medicine,
University of Basel, Switzerland, Summer 1961.

First Assistant in Anatomy, School of Medicine,
University of Lausanne, Switzerland, 1962-1965.

Assistant and Associate Professor of Anatomy,
School of Medicine, St. Louis University, St. Louis,
Missouri, 1965-1971.

Associate Professor of Anatomy, School of Medicine,
Georgetown University, Washington, D.C., 1971--

Member of :

REDACTED

Five Recent Publications:

1. Vidic, B., M.W. Rana and B.D. Bhagat. A reversible damage of the rat upper respiratory tract caused by cigarette smoking. Archs. Otolaryng. (AMA), in press.
2. Vidic, B. Structure and cytochemistry of the acinar cell in the rat maxillary gland. Am. J. Anat., 137:103-117, 1973.
3. Vidic, B., J.J. Taylor, M.W. Rana and B.D. Bhagat. The respiratory glandular system in the rat lateral nasal wall in normal and polluted environments. Anat. Anz., 130:83-85, 1972.
4. Vidic, B. and J.J. Taylor. The structure of the acinar cell and its relationship to the nerve terminals in the lateral nasal gland of the rat. Arch. Histol. Jap., 34:449-461, 1972.
5. Vidic, B.
The histochemical and microscopical differentiation of the respiratory glands around the maxillary sinus of the rat. Am. J. Anat., 132:491-514, 1971.

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BIOGRAPHICAL SKETCH

Paul Hamosh, M.D.

REDACTED

REDACTED

*Just the
address, not
the name*Graduate and Post-Graduate Education

- 1951-1957 Attended Medical School, Hebrew University, Hadassa Medical School, Jerusalem, Israel
- 1957-1963 Rotating internship and residency in Pathology, Internal Medicine and Chest Diseases
- 1963-1965 Fellow and Staff, Department of Medicine, B., "Ichilow" Municipal Hospital, Tel-Aviv, Israel
- 1966-1968 NIH Trainee in Cardio-Pulmonary Physiology, Department of Medicine, Georgetown University Medical School and Veterans Administration Hospital, Washington, D.C.

Professional Appointments

- 1968-1970 Lecturer in Physiology, Georgetown University Medical School
- 1970-1972 Assistant Professor of Medicine, George Washington University Medical School
- 1968-1970 Research Associate, Veterans Administration Hospital, Washington, D.C.
- 1970-1972 Director, Pulmonary Physiology Laboratory, Veterans Administration Hospital, Washington, D.C.
- 1972-date Assistant Professor of Physiology and Biophysics, Georgetown University Medical School
- 1973-date Assistant Professor of Medicine, Georgetown University Medical School
- 1973-date Senior Cancer Research Internist, Veterans Administration Hospital/National Cancer Institute, Washington, D.C. (Part-time)

Honors

- 1972 Clinical Investigator, Veterans Administration (declined)

Societies

REDACTED

Five Recent Publications

1. Hamosh, P., Da Silva, A.M.T. Postural hypoxemia and erythrocytosis in two non-obese patients without manifest lung disease. Am. J. Med. 55:80, 1973.
2. Da Silva, A.M.T., and Hamosh, P. The effect of smoking a single cigarette on the small airways. J. Appl. Physiol. 34:361, 1973.
3. Gacad, G., and Hamosh, P. The lung in ankylosing spondylitis. Am. Rev. Resp. Dis. 107:286, 1973.

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4. Hamosh, P. and Luchsinger, P.C. Respiratory mechanics and gas exchange in the squatting position. Am. Rev. Resp. Dis. 102:112, 1970.
5. Hamosh, P., Luchsinger, P.C. Maximum expiratory flow in isolated liquid-filled lungs. J. Appl. Physiol. 25:485, 1968.

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BIOGRAPHICAL SKETCH

Henry Yeager, Jr., M.D.

Personal Information

Place of Birth:

Date of Birth:

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Marital Status:

Address: Georgetown Univ. Hospital Home:
 3800 Reservoir Road, NW
 Washington, D.C. 20007
 (202) 625-7027

REDACTED

Education

Southern Methodist University (Phi Beta Kappa)
 Johns Hopkins University

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Postgraduate Education

Internship, Vanderbilt Hospital, Nashville, Tenn.	1957-1958
Residency in Internal Medicine, Parkland Memorial Hospital, Dallas, Texas	1958-1960
Fellowship in Arthritis, Dr. Morris Ziff, Southwestern Medical School, Dallas, Texas	1960-1961
Fellowship in Pulmonary Disease, Dr. Charles A. LeMaistre, Southwestern Medical School, Dallas, Texas	1963-1964
Fellowship in Pulmonary Disease, Dr. H.O. Sieker, Duke University School of Medicine, Durham, North Carolina	1967-1968

Specialty Board: Diplomate, American Board of Internal Medicine 1965

Professional Experience

Instructor, Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas	1964-1965
Clinical Instructor, Internal Medicine, University of Texas, Southwestern Medical School, Dallas, Tex.	1965-1967
Consultant Internist in Project HOPE, Nicaragua, Central America (Sept.-Nov.)	1966
Research Associate, V.A. Hospital, Washington, D.C.	1968-1970
Assistant Professor of Medicine, George Washington University School of Medicine, Washington, D.C.	1968-1970
Staff Physician, Medical Service, V.A. Hospital, Houston, Texas	1970-1972
Assistant Professor of Medicine, Baylor College of Medicine, Houston, Texas	1970-1972
Assistant Professor of Medicine, Georgetown University School of Medicine, Washington, D.C.	1972--

Medical License: Maryland, Texas and District of Columbia

Professional Societies

1960
 1960
 1963
 1967
 1971

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Military Service

U.S. Army, Captain in Medical Corps, Ft. Hood, Texas 1961-1963

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Five Recent Publications

1. Yeager, H., Jr. and Massaro, D. Glucose metabolism and glycoprotein synthesis in lung slices. J. Appl. Physiol. 32:477, 1972.
2. Yeager, H., Jr. Tracheobronchial secretions. Am. J. Med. 50:493, 1971.
3. Yeager, H., Jr., Massaro, D., and Massaro, G. Glycoprotein synthesis by the trachea. Am. Rev. Resp. Dis. 103:188, 1971.
4. Massaro, D., Massaro, G., Keleher, K., and Yeager, H., Jr. Alveolar cells: depression of protein synthesis during phagocytosis. Am. J. Physiol. 218:1533, 1970.
5. Yeager, H., Jr. Alveolar cells: depressant effect of cigarette smoke on protein synthesis. Proc. Soc. Exper. Biol. Med. 131:247, 1969.

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BIOGRAPHICAL SKETCH

Margit Hamosh, Ph.D.

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Graduate and Post-Graduate Education

REDACTED

Attended Hebrew University
M.Sc. in Microbiology
Doctoral Fellow, Department of Biochemistry, Hebrew
University Medical School
Ph.D. in Biochemistry
Postdoctoral work in Department of Biochemistry,
Hebrew University

Appointments

1964 Lecturer in Biochemistry, Hebrew University Medical
School
1965-date Visiting Scientist at National Institute of Arthritis,
Metabolism and Digestive Diseases

Five Recent Publications

1. M. Hamosh and R.O. Scow. Lingual lipase and its role in the digestion of dietary lipid. J. Clin. Invest. 52:88, 1973.
2. M. Hamosh and R.O. Scow. Lipoprotein lipase activity in guinea pig and rat milk. Biochim. Biophys. Acta 231:283, 1971.
3. Margit Hamosh and Robert O. Scow: Plasma triglyceride and lipoprotein lipase activity in pregnant and lactating rats. Nutrition, Proc. VIII International Congress on Nutrition, Prague 1969, Ed. J. Masek et al., Excerpta Medica, ICS No. 213, 207-209, 1970.
4. M. Hamosh, T.R. Clary, S.S. Chernick and R.O. Scow. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim. Biophys. Acta 210:473, 1970.
5. M. Hamosh, M. Lesch, J. Baron and S. Kaufman: Enhanced protein synthesis in a cell-free system from hypertrophied skeletal muscle. Science 157:935, 1967.

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14. First year budget:

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s);
even if no salary requested)

	% time	Amount
Dr. Branislav Vidic	25	--
Dr. Paul Hamosh	15	--
Dr. Henry Yeager, Jr.	Consultant	--
Dr. Margit Hamosh	Consultant	--
Research Assistant (to be hired)* (M.S. or Ph.D.)	100	R

Technical Technician *	100	R
Administrative Assistant (data processing)*	25	

* 11% Fringe Benefits included.

Sub-Total for A

B. Consumable supplies (by major categories)

1. Experimental animals (purchase and maint.)	2,500.
2. Chemicals for ultrastructure	750.
3. Radioactive isotopes	750.
4. Chemicals for biochemistry	1,250.
5. Photographic supplies	1,500.
6. Glassware, miscellaneous	500.

Sub Total for B 7,250.

C. Other expenses (itemize)

Consultant, maintenance of equipment	1,000.
Data processing, computer time	500.
Travel	1,000.
Publication	500.

Sub-Total for C 3,000.

Running Total of A + B + C 33,250.

D. Permanent equipment (itemize)

Estimated cost of exposure facility	2,500.
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Sub-Total for D 2,500.

E. Indirect costs (15% of A+B+C)

E 4,988.

Total request \$40,738.

15. Estimated future requirements.

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R 7,975.	3,300.	--	5,486.	42,061.	
Year 3	8,773.	3,630.	--	6,034.	46,267	

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5.

16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
A reparable damage of the maxillary epithelium and gland of the rat caused by cigarette smoking.	PHS grant RR5360-11	1,000.	9/30/71-8/31/73
The effect of whole cigarette smoke on the oral tissue.	GRS grant. Georgetown Univ. Dental School	800.	3/14/72-8/31/73
Ultrastructure and metabolism of isolated perfused lung.	Washington Heart Assoc. grant 3-282-777	7,532.	7/1/73-6/30/74
Surfactant metabolism as a function of ventilation	Washington Heart Assoc. grant 3-287-785	7,528.	1/1/73-12/31/73
Effect of mechanical stress on the elastic properties of the lung in papain induced emphysema	GRS-NIH 3302-113 Georgetown Univ. Med. School	2,500.	1/1/73-12/31/73
Quantitation of lung cancer on chest films by computer	Contract with V.A.	10,800.	1/1/73-12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Computerized chest X-ray followup in cancer therapy	National Cancer Inst.	80,460.	1/1/74-12/31/76
Federic lung development program project	National Heart and Lung Institute	Approx. 200,000.	7/1/74-6/30/79

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Branislav Vidic, S.D.Signature [Signature] Date 7/31/73Telephone R Area Code Number Extension

Checks payable to

Georgetown University

Mailing address for checks Sam A. Kimble
Georgetown University
37th and O Streets, N.W.

Washington, D.C. 20007

Responsible officer of institution

Typed Name Guerry R. Smith

Acting
 Title Administrator, Sponsored Programs

Signature [Signature] Date 7/31/73Telephone R Area Code Number Extension

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